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NSF Engineering Research Center for Biorenewable Chemicals

CBiRC
NSF Engineering Research Center for Biorenewable Chemicals

FIFTH ANNUAL REPORT

VOLUME II | APRIL 10, 2013

Dr. Brent Shanks, Director
Dr. Basil Nikolau, Deputy Director

Core Partner Institutions

Iowa State University (Lead)

Pennsylvania State University

Rice University

University of California, Irvine

University of New Mexico

University of Virginia

University of Wisconsin, Madison

A National Science Foundation Engineering Research Center since 2008
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Table of Contents

List of Projects

Faculty Investigators	Department	Institution
Thrust 1 — New Biocatalysts for Pathway Engineering		
CENTER-CONTROLLED (CORE) PROJECTS		
T1.1 – 3-Ketoacyl-ACP Synthase: Characterization of Novel Biocatalysts (3-ketoacyl Synthases) for Diversifying FAS/PKS Metabolic Pathways		
Joseph P. Noel *(Lead)*	Jack H. Skirball Center for Chemical Biology & Proteomics	Salk Institute for Biological Studies
Adam Barb	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Eran Pichersky	Molecular, Cellular & Developmental Biology	University of Michigan
Peter J. Reilly	Chemical & Biological Engineering	Iowa State University
Eve S. Wurtele	Genetics, Development & Cell Biology	Iowa State University
T1.2 – Acetoacetyl-CoA: Use of *Escherichia coli* for the Production of Molecules Functionalized for Chemical Synthesis		
Thomas A. Bobik *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
T1.3 – Acetyl-CoA/Propionyl-CoA Synthetase: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Peter J. Reilly	Chemical & Biological Engineering	Iowa State University
T1.4 – Acyl-CoA Carboxylases: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Adam Barb	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Peter J. Reilly	Chemical & Biological Engineering	Iowa State University
Eve S. Wurtele	Genetics, Development & Cell Biology	Iowa State University

T1.5 – Methylketone Synthase/Thioesterase: Development of Methylketone Synthase Enzyme Adapted for the Production of Short-Chain Methylketones		
Eran Pichersky *(Lead)*	Molecular, Cellular & Developmental Biology	University of Michigan
Joseph P. Noel	Jack H. Skirball Center for Chemical Biology & Proteomics	Salk Institute for Biological Studies
Peter J. Reilly	Chemical & Biological Engineering	Iowa State University
T1.6 – Thioesterases: Characterization of Novel Biocatalysts (Thioesterases) for Diversifying FAS/PKS Metabolic Pathways		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Peter J. Reilly	Chemical & Biological Engineering	Iowa State University
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine
Marna Yandeau-Nelson	Biochemistry, Biophysics & Molecular Biology	Iowa State University
02-13S2 - Structural and Substrate-binding Studies of KASIII Enzymes to Understand Their Evolutionary Relationship with PKS Enzymes (*Student-led Research Grant*)		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Joseph P. Noel	Jack H. Skirball Center for Chemical Biology & Proteomics	Salk Institute for Biological Studies
31-12F1 – Combinatorial Integration of Novel Biocatalysts that Intercept Fatty Acid Synthase to form Innovative Bi-functional Carboxylic Acids (*IAB Seed Project*)		
Marna Yandeau-Nelson *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
ASSOCIATED PROJECTS		
Biosynthesis of Alkamides – Experimental Modeling of a Modular Secondary Metabolic Pathway *National Science Foundation*		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Characterization of Biocatalysts for Novel Production Platforms for Diverse Bi-functional Precursors of Polymers and Surfactants *U.S. Department of Commerce (Translational Research)*		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University

Coenzyme B12-dependent 1,2-propanediol Degradation in *Salmonella* *National Science Foundation*		
Thomas A. Bobik *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
I-Corps: Novel Bio-Based Chemical Feedstocks for the Polymer Industry *National Science Foundation (Translational Research)*		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Peter L. Keeling	ERC for Biorenewable Chemicals	Iowa State University
Mechanistic and Structural Basis for Plant Metabolic Evolution *Howard Hughes Medical Institute*		
Joseph P. Noel *(Lead)*	Jack H. Skirball Center for Chemical Biology & Proteomics	Salk Institute for Biological Studies
Metabolomics: A Functional Genomics Tool for Deciphering Functions of *Arabidopsis* Genes in the Context of Metabolic and Regulatory Networks *National Science Foundation*		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Julie A. Dickerson	Electrical & Computer Engineering	Iowa State University
Eve S. Wurtele	Genetics, Development & Cell Biology	Iowa State University
SoLysis: A Start-up Focused on Novel Biocatalysts for the Production Platforms of Diverse Fatty Acid Products *U.S. Department of Commerce (Translational Research)*		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Peter L. Keeling	ERC for Biorenewable Chemicals	Iowa State University
Marna Yandeau-Nelson	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Uncovering Novel Signaling Interactions in Plant Metabolic Networks *National Science Foundation*		
Eve S. Wurtele *(Lead)*	Genetics, Development & Cell Biology	Iowa State University
Ling Li	Genetics, Development & Cell Biology	Iowa State University

Faculty Investigators	Department	Institution
Thrust 2 — Microbial Metabolic Engineering		
CENTER-CONTROLLED (CORE) PROJECTS		
T2.1A – Strain Construction and Optimization in *E. coli*		
Ka-Yiu San *(Lead)*	Bioengineering	W. M. Rice University
Ramon Gonzalez	Chemical & Biomolecular Engineering	W. M. Rice University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
T2.1B – Strain Construction and Optimization in *S. cerevisiae*		
Nancy A. Da Silva (*Lead*)	Chemical Engineering & Materials Science	University of California – Irvine
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine
Zengyi Shao	Chemical & Biological Engineering	Iowa State University
T2.2A – Strain Characterization and Optimization in *E. coli*		
Ka-Yiu San *(Lead)*	Bioengineering	W. M. Rice University
Ramon Gonzalez	Chemical & Biomolecular Engineering	W. M. Rice University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
T2.2B – Strain Characterization and Optimization in *S. cerevisiae*		
Nancy A. Da Silva (*Lead*)	Chemical Engineering & Materials Science	University of California – Irvine
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine
Zengyi Shao	Chemical & Biological Engineering	Iowa State University
T2.3A – Omics Experiments in *E. coli*		
Ramon Gonzalez (*Lead*)	Chemical & Biomolecular Engineering	W. M. Rice University
Julie A. Dickerson	Electrical & Computer Engineering	Iowa State University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University

T2.3B – Omics Experiments in *S. cerevisiae*		
Laura R. Jarboe (*Lead*)	Chemical & Biological Engineering	Iowa State University
Nancy A. Da Silva	Chemical Engineering & Materials Science	University of California – Irvine
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine
T2.4A – Flux Analysis in *E. coli*		
Jacqueline V. Shanks (*Lead*)	Chemical & Biological Engineering	Iowa State University
Ramon Gonzalez	Chemical & Biomolecular Engineering	W. M. Rice University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
Costas D. Maranas	Chemical Engineering	Pennsylvania State University
Ka-Yiu San	Bioengineering	W. M. Rice University
T2.4B – Flux Analysis in *S. cerevisiae*		
Jacqueline V. Shanks (*Lead*)	Chemical & Biological Engineering	Iowa State University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
Costas D. Maranas	Chemical Engineering	Pennsylvania State University
T2.5A – Bioinformatics in *E. coli*		
Julie A. Dickerson (*Lead*)	Electrical & Computer Engineering	Iowa State University
Ramon Gonzalez	Chemical & Biomolecular Engineering	W. M. Rice University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
Ka-Yiu San	Bioengineering	W. M. Rice University
Jacqueline V. Shanks	Chemical & Biological Engineering	Iowa State University
T2.5B – Bioinformatics in *S. cerevisiae*		
Eve S. Wurtele (*Lead*)	Genetics, Development & Cell Biology	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine
Jacqueline V. Shanks	Chemical & Biological Engineering	Iowa State University

T2.6A – Beta-oxidation Pathway Reversal in *E. coli*		
Ramon Gonzalez (*Lead*)	Chemical & Biomolecular Engineering	W. M. Rice University
02-12F1 – 13C Metabolic Flux Analysis Based Fluxomics Comparison Between *Saccharomyces cerevisiae* and Oleaginous Yeast *Yarrowia lipolytica* *(Student-led Research Grant)*		
Jacqueline V. Shanks (*Lead*)	Chemical & Biological Engineering	Iowa State University
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine
32-12F2 - Functional Genomics Profiling of the Oleaginous Yeast Stress Response *(IAB Seed Project)*		
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine
ASSOCIATED PROJECTS		
A Native Pathway for the Production of n-butanol in *Eschericia coli*: A New Paradigm for Synthetic Biology *National Science Foundation*		
Ramon Gonzalez (*Lead*)	Chemical & Biomolecular Engineering	W. M. Rice University
A Robust Platform for Reconstituting and Engineering Iterative Megasynthases *National Institutes of Health*		
Nancy A. Da Silva	Chemical Engineering & Materials Science	University of California – Irvine
Biological Utilization of Thermolytic Substrates by Bacteria and Microalgae *National Science Foundation*		
Laura R. Jarboe *(Lead)*	Chemical & Biological Engineering	Iowa State University
Collaborative Research: Metabolic Engineering of Terpenoid Indole Alkaloids Using Transcriptional Regulators in *C. roseus* Hairy Roots *National Science Foundation*		
Jacqueline V. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
Efficient Synthesis of Hydrocarbons Using an Engineered Reversal of the β-oxidation Cycle: A New Paradigm for the Production of Advanced Biofuels *National Science Foundation*		
Ramon Gonzalez *(Lead)*	Chemical & Biomolecular Engineering	W. M. Rice University
EFRI-HyBi: Bioengineering a System for the Direct Production of Biological Hydrocarbons for Biofuels *National Science Foundation*		
Jacqueline V. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University

Energy Efficient Cultivation of Microalgae and Simultaneous Separation of Products Using a Novel Taylor Vortex Reactor-Separator *ConocoPhillips Company*		
Jacqueline V. Shanks	Chemical & Biological Engineering	Iowa State University
Engineering Yeast Consortia for Surface-Display of Complex Cellulosome Structure: A Consolidated Bioprocessing Approach from Cellulosic Biomass to Ethanol *National Science Foundation*		
Nancy A. Da Silva	Chemical Engineering & Materials Science	University of California – Irvine
Evaluate and Identify Metabolic Control Points Determining Assimilate Partitioning in Developing Seed *Pioneer Hi-Bred International, Inc.*		
Jacqueline V. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
Generation of Biofuels from Abundant Non-digestible Oilseed Components *National Science Foundation*		
Ka-Yiu San *(Lead)*	Bioengineering	W. M. Rice University
Genetic and Environmental Factors Driving *E. coli* Attachment to Particles in Streams *National Science Foundation*		
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
Lignocellulosic Biomass Conversion to Infrastructure Compatible Fuel, Products and Power *U.S. Department of Agriculture (Translational Research)*		
Ka-Yiu San	Bioengineering	W. M. Rice University
Mass Spectrometric Imaging of Plant Metabolites *U. S. Department of Energy*		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
MRI: Acquisition of a Tandem (IT-TOF) Mass Spectrometer System for Biological Research and Application *National Science Foundation*		
Ka-Yiu San *(Lead)*	Bioengineering	W. M. Rice University
Small Business ERC Collaborative Opportunity to Develop a Biomass Conversion to Fatty Acids Platform *National Science Foundation (Translational Research)*		
Ka-Yiu San *(Lead)*	Bioengineering	W. M. Rice University
Use of Systems Biology Approaches to Develop Advanced Biofuel-Synthesizing Cyanobacterial Strains *U.S. Department of Energy*		
Costas D. Maranas	Chemical Engineering	Pennsylvania State University

Faculty Investigators	Department	Institution
Thrust 3 — Chemical Catalyst Design		
CENTER-CONTROLLED (CORE) PROJECTS		
T3.2 – Selective Dehydration of Model Compounds		
Brent H. Shanks (*Lead*)	Chemical & Biological Engineering	Iowa State University
Abhaya K. Datye	Chemical & Nuclear Engineering	University of New Mexico
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
T3.3 – Deoxygenation of Fatty Acids		
Robert J. Davis *(Lead)*	Chemical Engineering	University of Virginia
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
George A. Kraus	Chemistry	Iowa State University
Matthew Neurock	Chemical Engineering	University of Virginia
T3.5 – Ring Opening Reactions		
James A. Dumesic *(Lead)*	Chemical Engineering	University of Wisconsin – Madison
Abhaya K. Datye	Chemical & Nuclear Engineering	University of New Mexico
Robert J. Davis	Chemical Engineering	University of Virginia
Matthew Neurock	Chemical Engineering	University of Virginia
T3.7 – Hydrothermally Stable Catalysts and Catalyst Supports		
Abhaya K. Datye *(Lead)*	Chemical & Nuclear Engineering	University of New Mexico
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
Klaus Schmidt-Rohr	Chemistry	Iowa State University
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
Jean-Philippe Tessonnier	Chemical & Biological Engineering	Iowa State University
T3.9 – Pyrone Conversions		
George A. Kraus (*Lead*)	Chemistry	Iowa State University
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison

T3.10 – Selective Oxidation to Di-acids		
Robert J. Davis *(Lead)*	Chemical Engineering	University of Virginia
Matthew Neurock	Chemical Engineering	University of Virginia
T3.11 – Migration of Functional Groups		
L. Keith Woo (*Lead*)	Chemistry	Iowa State University
02-12F2 - Production of Monomers for Nylon-6,6 from Biorenewable Sugars *(Student-led Research Grant)*		
Brent H. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
Robert J. Davis	Chemical Engineering	University of Virginia
02-12F3 – Applicability of Novel Heterogeneous Palladium Catalysts in Industrially-Relevant Organic Transformations *(Student-led Research Grant)*		
George A. Kraus *(Lead)*	Chemistry	Iowa State University
Abhaya K. Datye	Chemical & Nuclear Engineering	University of New Mexico
02-12F5 - Design of Carbon Nanocoated Oxide Supports without Mass Transfer Limitations for Production of alpha Olefins from Carboxylic Acid *(Student-led Research Grant)*		
Robert J. Davis *(Lead)*	Chemical Engineering	University of Virginia
Abhaya K. Datye	Chemical & Nuclear Engineering	University of New Mexico
02-13S1 - Rapid, High Throughput Identification and Quantification of Carbohydrates and Their Derivatives Using UPLC-PDA-ELSD *(Student-led Research Grant)*		
Jean-Philippe Tessonnier *(Lead)*	Chemical & Biological Engineering	Iowa State University
Laura Jarboe	Chemical & Biological Engineering	Iowa State University
Adam Okerlund	Center for Biorenewable Chemicals	Iowa State University
33-12F3 - High Throughput Facility for Reaction Kinetics Measurements in the Development of Amino-Acid Tolerant Heterogeneous Catalysts *(IAB Seed Project)*		
James A. Dumesic *(Lead)*	Chemical Engineering	University of Wisconsin – Madison
Nancy A. Da Silva	Chemical Engineering & Materials Science	University of California – Irvine
Abhaya K. Datye	Chemical & Nuclear Engineering	University of New Mexico

SPONSORED PROJECTS		
ERC – Small Business: Commercialization of Furanic-based Biorenewable Chemicals *National Science Foundation (Translational Research)*		
Adam L. Okerlund *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
Grow Iowa Values Fund: Catalytic Conversion Platform for Furan Derivatives *Iowa Board of Regents (Translational Research)*		
Peter L. Keeling *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
Adam L. Okerlund	ERC for Biorenewable Chemicals	Iowa State University
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
Proof of Concept Initiative: Biobased Production of Terephthalic Acid *U.S. Department of Commerce (Translational Research)*		
George A. Kraus *(Lead)*	Chemistry	Iowa State University
Peter L. Keeling	ERC for Biorenewable Chemicals	Iowa State University
Adam L. Okerlund	ERC for Biorenewable Chemicals	Iowa State University
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
Proprietary Project – Title Undisclosed *Chevron Phillips Chemical Company, LLC (Translational Research)*		
Brent H. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
Adam L. Okerlund	ERC for Biorenewable Chemicals	Iowa State University
Selective Dehydration of Multifunctional Substrates *National Center for Agricultural Utilization Research (NCAUR), USDA*		
Brent H. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
George A. Kraus	Chemistry	Iowa State University
ASSOCIATED PROJECTS		
Catalytic Reactivity at the Metal-Solution Interface *National Science Foundation*		
Robert J. Davis *(Lead)*	Chemical Engineering	University of Virginia

GOALI: Understanding Self-assembly of Noble Metal Alloys for Ultra Low Temperature Oxidation Catalysis *National Science Foundation*		
Abhaya K. Datye *(Lead)*	Chemical & Nuclear Engineering	University of New Mexico
Green Catalysis *National Science Foundation*		
L. Keith Woo (*Lead*)	Chemistry	Iowa State University
Materials for Energy Conversion *U. S. Department of Energy*		
Abhaya K. Datye	Chemical & Nuclear Engineering	University of New Mexico
National Advanced Biofuels Consortium *U. S. Department of Energy*		
Brent H. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels, Chemicals and Materials[3] *National Science Foundation*		
Abhaya K. Datye (*Lead*)	Chemical & Nuclear Engineering	University of New Mexico
Ib Chorkendorff	Physics	Technical University of Denmark
Robert J. Davis	Chemical Engineering	University of Virginia
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
George A. Kraus	Chemistry	Iowa State University
Dmitry Murzin	Chemical Engineering	Abo Akademi University
Matthew Neurock	Chemical Engineering	University of Virginia
Hans Niemantsverdriet	Schuit Institute of Catalysis	Eindhoven University of Technology
Robert Schlögl	Inorganic Chemistry	Fritz Haber Institute of the Max Planck Society
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
Selective Hydrogenation of Oxygenates *Engineering and Physical Sciences Research Council (United Kingdom)*		
Matthew Neurock	Chemical Engineering	University of Virginia

Structure and Function of Supported Base Catalysts *U. S. Department of Energy*		
Robert J. Davis (*Lead*)	Chemical Engineering	University of Virginia
The Science and Engineering of Durable Ultra-Low Platinum Group Metal Catalysts *Los Alamos National Labs*		
Abhaya K. Datye *(Lead)*	Chemical & Nuclear Engineering	University of New Mexico

Faculty Investigators	Department	Institution
Research Support – Life Cycle Assessment (LCA)		
CENTER-CONTROLLED (CORE) PROJECTS		
Techno-Economic Analysis of Making Hydrocarbons from Biomass-Derived Sugars		
Robert P. Anex *(Lead)*	Biological Systems Engineering	University of Wisconsin – Madison
D. Raj Raman	Agricultural & Biosystems Engineering	Iowa State University
02-12F4 – Determining Different Reaction Engineering Factors that Affect the Economic Feasibility of the Production of alpha Olefins from Carboxylic Acids through Techno-economic Analysis *(Student-led Research Grant)*		
Robert J. Davis (*Lead*)	Chemical Engineering	University of Virginia
Robert P. Anex	Biological Systems Engineering	University of Wisconsin – Madison
ASSOCIATED PROJECTS		
A Regional Program for Production of Multiple Agricultural Feedstocks and Processing to Biofuels and Biobased Chemicals *U.S. Department of Agriculture*		
Robert P. Anex	Biological Systems Engineering	University of Wisconsin – Madison

Faculty Investigators	Department	Institution
Pre-College Education		
CENTER-CONTROLLED (CORE) PROJECTS		
Pre-College Learning Modules		
Adah Leshem *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
D. Raj Raman	Agricultural & Biosystems Engineering	Iowa State University
Teacher Professional Development (RET and Summer Academy Programs)		
Adah Leshem *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
Peter L. Keeling	ERC for Biorenewable Chemicals	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
D. Raj Raman	Agricultural & Biosystems Engineering	Iowa State University
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
Young Engineers Program		
Adah Leshem *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
SPONSORED PROJECTS		
GK-12: *Symbi*, Iowa's GK-12 Program – Growing Iowa's Scientists for a Greener Tomorrow *National Science Foundation*		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Adah Leshem	ERC for Biorenewable Chemicals	Iowa State University
D. Raj Raman	Agricultural & Biosystems Engineering	Iowa State University
Integration of STEM through Problem-based Learning[1] *Des Moines Public Schools*		
Adah Leshem *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University

ASSOCIATED PROJECTS		
Iowa EPSCoR: Harnessing Energy Flows in the Biosphere to Build Sustainable Energy Systems *National Science Foundation*		
Adah Leshem	ERC for Biorenewable Chemicals	Iowa State University
Meta!Blast: An Immersive Interactive Learning Module for Cell Biology *National Institutes of Health*		
Eve S. Wurtele (*Lead*)	Genetics, Development & Cell Biology	Iowa State University
Adah Leshem	ERC for Biorenewable Chemicals	Iowa State University
Plants in Society *National Science Foundation*		
Adah Leshem	ERC for Biorenewable Chemicals	Iowa State University

Faculty Investigators	Department	Institution
University (Graduate and Undergraduate) Education		
CENTER-CONTROLLED (CORE) PROJECTS		
CBiRC Graduate Minor and Graduate Certificate Programs		
D. Raj Raman *(Lead)*	Agricultural & Biosystems Engineering	Iowa State University
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
CBiRC Research Experience for Undergraduates (REU) Program		
D. Raj Raman *(Lead)*	Agricultural & Biosystems Engineering	Iowa State University
Thomas A. Bobik	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Robert J. Davis	Chemical Engineering	University of Virginia
Ramon Gonzalez	Chemical & Biomolecular Engineering	W. M. Rice University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
George A. Kraus	Chemistry	Iowa State University
Matthew Neurock	Chemical Engineering	University of Virginia
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Ka-Yiu San	Bioengineering	W. M. Rice University

Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
Jacqueline V. Shanks	Chemical & Biological Engineering	Iowa State University
L. Keith Woo	Chemistry	Iowa State University
SPONSORED PROJECTS		
EFRI-HyBi: Bioengineering a System for the Direct Production of Biological Hydrocarbons for Biofuels *(REU Supplement)*[2] *National Science Foundation*		
Jacqueline V. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
D. Raj Raman	Agricultural & Biosystems Engineering	Iowa State University
Iowa State Coleman Faculty Entrepreneurship Fellow: Development of BR C 507X, *Entrepreneurship in Biorenewable Chemicals* *Coleman Foundation (channeled through the ISU Foundation)*		
Peter L. Keeling *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
D. Raj Raman	Agricultural & Biosystems Engineering	Iowa State University
ASSOCIATED PROJECTS		
IINspire LSAMP – An Alliance Modeling How to Broaden Participation in Changing Midwest Demographics *National Science Foundation*		
Krishna S. Athreya	ERC for Biorenewable Chemicals	Iowa State University
UCI Biomedical Informatics Training Undergraduate Summer Research (BIT-SR) Program *National Institutes of Health*		
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine

Faculty Investigators	Department	Institution
International Education		
ASSOCIATED PROJECTS		
PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels, Chemicals and Materials[3] *National Science Foundation*		
Abhaya K. Datye (*Lead*)	Chemical & Nuclear Engineering	University of New Mexico
Ib Chorkendorff	Physics	Technical University of Denmark
Robert J. Davis	Chemical Engineering	University of Virginia
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
George A. Kraus	Chemistry	Iowa State University
Dmitry Murzin	Chemical Engineering	Abo Akademi University
Matthew Neurock	Chemical Engineering	University of Virginia
Hans Niemantsverdriet	Schuit Institute of Catalysis	Eindhoven University of Technology
Robert Schlögl	Inorganic Chemistry	Fritz Haber Institute of the Max Planck Society
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University

Faculty Investigators	Department	Institution
Innovation and Entrepreneurship		
SPONSORED PROJECTS		
Biobased Foundry *College of Engineering, Iowa State University*		
Peter L. Keeling (*Lead*)	ERC for Biorenewable Chemicals	Iowa State University
Regents Innovation Fund: Biobased Foundry *Iowa Board of Regents*		
Peter L. Keeling (*Lead*)	ERC for Biorenewable Chemicals	Iowa State University

Footnotes:

1. See Project Summary entitled "Teacher Professional Development" under Pre-College Education.
2. See Project Summary entitled "Research Experience for Undergraduate (REU) Program" under University Education Program.
3. See Project Summary entitled "PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels, Chemicals and Materials," under International Education Program.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T1.1 – 3-Ketoacyl-ACP Synthase -** Characterization of Novel Biocatalysts for Diversifying FAS/PKS Metabolic Pathways

Thrust: Research Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Joseph P. Noel	**02/14/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Joseph P. Noel, Salk Institute for Biological Studies
Other Faculty: Basil J. Nikolau, Peter J. Reilly, Iowa State University; Eran Pichersky, University of Michigan
Postdocs: Kate Woods, Charles Stewart and Yongxia Guo, Salk Institute
Graduate Students: David Cantú, Yingfei Chen, Huanan Jin and Shivani Garg, Iowa State University
Undergraduate Students: Bing O'Dowd (University of California, San Diego), Adam Dobson (University of Pittsburgh), Aidan Gilchrist (University of Massachusetts Amherst), Phillip Guichet (University Of San Diego), Erina He (University Of California, San Diego), Kyle Merchant (University of California, San Diego), Salk Institute, and Morgan Becker, Iowa State University
High School Students: Alyssa Herperger (Canyon Crest Academy), Salk Institute
Other Personnel: Marna Yandeau-Nelson, Iowa State University, Gordon Louie and Marianne Bowman, The Salk Institute

Statement of Project Goals

The overarching goal of this project is to identify and characterize novel biocatalysts from plant and microbial polyketide synthase (PKS) systems for the purpose of diversifying the malonyl-CoA pools of *E. coli* and the yeast *Saccharomyces cerevisiae*. This project targets biocatalysts that will expand substrates used in the carbon-carbon and carbon-oxygen bond forming reactions of fatty acid and polyketide biosynthetic cycles. Specifically, we focus on (1) the 3-ketoacyl-ACP synthase III (KAS III) family of condensing enzymes and (2) the evolutionarily related type III PKSs commonly found in plants. The objective of project goal 1 is to clone, express, biochemically characterize, and when successful, crystallize and solve the atomic resolution 3D structures of orthologs of KAS IIIs from hosts with unconventional substrate selection (acetyl-CoA being conventional), to create new metabolic intermediates. The objective of project goal 2 is to create a platform to evaluate structure-based mutant libraries of *Gerbera hybrida* 2-pyrone synthase (2-PS) and evolutionarily related plant type III polyketide synthases (PKS IIIs) such as chalcone, stilbene, orcinol and bibenzyl synthases that employ one molecule of acetyl-CoA and two molecules of malonyl-CoA to biosynthesize 6-methyl-4-hydroxy-2-pyrone (2-PY), one of our lead test beds, or orcinol (5-methylbenzene-1,3-diol), a developing test bed. Given our atomic resolution structural knowledge of 2-PS and related type IIIs, we will

create mutant libraries centered on the active site (focused) and random libraries spread throughout the protein to uncover catalytically more efficient enzymes for 2-PY production. A second objective is to employ rationally designed fusion proteins of type III PKSs with the anabolic thiolases to create a localized pool of Acetoacetyl-CoA to bypass the need for at least one malonyl-CoA.

Using a combination of atomic resolution protein x-ray crystallography, site-directed and combinatorial mutagenesis and high-throughput in vitro biochemistry, we will rationally modulate the efficiency and specificity of all project goal targets for the production of short-chain keto-containing products for downstream processing or as test bed end products. By the end of Year 6, our goal is to engineer at least one additional biocatalysts that efficiently accepts an alternative starter unit (Thrust 1) and produces a reactive intermediate or end product in a microbial fermentation system (Thrust 2) that, upon scale-up and isolation, is delivered to Thrust 3 for large-scale chemical processing.

Project's Role in Center's Strategic Plan

A diverse collection of KASIII enzymes occurs in different biological systems that utilize different acyl-CoA substrates in this reaction. These ultimately add functionalities at the omega-end of the fatty acid products. The goal of this project is two-fold: 1) Find and characterize the molecular details of the nature of these KASIII orthologs that display different substrate specificities; and 2) based on the understanding of the design principle of these KASIII enzymes, create by mutagenesis novel KASIII orthologs that display distinct substrate specificities.

The atomic resolution crystal structures of several type III PKSs including the *G. hybrida* 2-PS with substrates and intermediates bound have been solved. The structures along with their quantitative biochemical characterization affords an atomic resolution map to greatly expand our mutant libraries of 2-PS to alter starter molecule loading from acetyl-CoA to other CoA-linked starters including succinyl-CoA. This will diverse the output of the enzyme affording ready access to succinyl-CoA pools in Thrust 2 and bifunctional pyrones for use in Thrust 3. This engineering approach is being supplemented by a new computational tool set developed in the Noel lab that makes use of Minimal Models of Multidimensional Computations. In short, we maximize the noise entropy of the system (mutations linked to a quantitative change in a biochemical activity) subject to constraints on the input/output moments, resulting in the response function that agrees with our limited knowledge and is maximally uncommitted toward everything else. Preliminary use of these computational tools has allowed us to detect co-varying relationships between amino acid positions and increases in catalytic activity and reactant specificity.

Fundamental Barriers and Methodologies

- Development of specific substrates (e.g. malonyl ACP) for the KAS III spectrophotometric enzymatic assay is a bottleneck due to their poor stability.
- The unavailability of KAS III gene sequences from organisms that synthesize halogenated fatty acids has limited the work to KAS III sequences from organisms that make branched chain or hydroxy-fatty acids.
- Lack of more catalytically efficient PKS IIIs for the practical production of the pyrone test bed products integral to the aims of Thrusts 1-3.
- Need for computational tools for constructing a model of the system using limited knowledge of

the correlations between inputs and outputs that can eliminate the imposition of implicit assumptions and biases leading to a mischaracterization of the computations leading to predictable engineering of catalytically efficient and specific KAS IIIs and type III PKSs.

Achievements

From 3/1/2012 to 2/29/2013

- NMR study to determine the effect of specific mutations on KASIII substrate specificity was extended to *B. subtilis* KASIIIb. Similar to the first experiment described in the achievements of 2012-13, single mutants were created for *B. subtilis* KASIIIb (W221V and V226L) and a double mutant (W221V_V226L) was also constructed to resemble the situation in the *E. coli* KASIII at those two sites. The effect of these mutations on the substrate specificity of *B. subtilis* KASIIIb was examined using STD NMR experiments, and results were compared with those of *E. coli* KASIII and *B. subtilis* KASIIIa.
- Competition binding experiments were performed to reveal relative affinities of ligands to KASIII enzymes, wherein concentration of acetyl CoA was kept constant and isobutyryl CoA was titrated at increasing concentrations to see if it replaces the former ligand.
- For *B. subtilis* KASIIIa, to determine the competition between acetyl CoA and isobutyryl CoA, we titrated increasing amounts of isobutyryl CoA, while acetyl CoA was held at a constant concentration. The results show that isobutyryl CoA was the preferred substrate for *B. subtilis* KASIIIa.
- Similar results were obtained for competition binding experiments with *B. subtilis* KASIIIb, where isobutyryl CoA was found to be the preferred substrate for *B. subtilis* KASIIIb.
- In addition to the seven KASIII genes cloned and purified in last year, we cloned eleven KASIII genes from a total of thirty genes that were synthesized previously (one KASIII gene from *Thermus aquaticus*, three for *Capnocytophaga gingivalis*, one from *Nocardiopsis dassonvellei*, two from *Brevibacterium linens*, three from *Myxococcus Xanthus*, one from *Methylosinus trichosporium*).
- Out of these eleven cloned genes, we successfully purified five KASIII proteins (three from *Capnocytophaga gingivalis,* two from *Brevibacterium linens,* one from *Methylosinus trichosporium).*
- Simultaneously, eleven KASIII genes were characterized *in vivo* using a *B. subtilis* FabH deletion mutant.
- We explored the commercial potential of KASIII technology to make bi-functional fatty acids, and developed a business model as a part of the NSF I-Corps Award.
- Cloned the first plant orcinol synthase (OS) (plant type III PKS) from the flowers of apricot trees. Apricot OS like 2-PS, employs acetyl-CoA for chain initiation and malonyl-CoA but terminates chain extension through a carbon-carbon bond forming Claisen condensation.
- Identified several sets of surface exposed and partially exposed Cys residues in 2-PS that when mutated to Ser or Ala resulted in enhanced stability of 2-PS during fermentation in yeast and a concomitant substantial increase in pyrone production.
- Identified additional active site residues in 2-PS that further restrict active site volume and increase specificity for pyrone biosynthesis.
- Implemented a first principles based computational tool borrowed from computational neurobiology to quantitatively link specific sets of mutants as single sites, pairs, triplet or more that significantly modulate catalytic activity and/or reactant specificity.

Other Relevant Work

Ketoacyl-ACP synthases from other organisms are being studied by other groups outside the ERC. For example, the structures of FabH from *S. aureus* and *S. pneumoniae* have been determined and proven useful in homology modeling. Those projects are focused on understanding the structure of these enzymes and developing antibiotics that can target these enzymes, which form a critical part of FAS system in bacteria. However, this project on KAS in the ERC not only aims at understanding the structural basis for differences in substrate specificities of KAS enzymes, but also aims at modifying their structures to yield novel biocatalysts useful for the chemical industry. Began further structure-function analysis of Biphenyl Synthase (BIS) from apple, another type III PKS with favorable substrate and product specificities. BIS is a homolog of 2-PS. Both BIS and 2-PS can use benzoyl-CoA as substrate; albeit benzoyl-CoA is an undesirable activity for 2-PS. We characterized the kinetics of BIS as a foundation for rational engineering to eliminate, or at least temper, benzoyl-CoA binding out of 2-PS and convert a BIS into a functional 2-PS. Moreover, we have a 1.1 Å structure of BIS bound to a variety of substrate, intermediate and product analogs providing us with the best structural model to date for any type III PKS.

Plans for the Next Year

- Biophysical and enzymological characterization of 12 more diverse KASIII enzymes identified by phylogenetic analyses.
- Determination of activity of newly cloned KASIII enzymes with non-endogenous substrates such as hydroxyl acyl CoA (e.g. 3-hydroxy butyryl CoA) and aromatic acyl CoA (e.g. benzoyl CoA).
- Identify additional key residues that are responsible for substrate specificities of KASIII enzymes: possible structure determination and mutagenesis (site-directed or random) of interesting candidates to create novel KASIII orthologs with ability to make substituted fatty acids.
- Structure determination of mutants of 2-PS by biophysical methods (e.g., x-ray crystallography).
- Expansion and recombination of more efficient 2-PS mutants to increase 2-PY production via an increase in kcat or a decrease in the non-productive malonyl CoA decarboxylation reaction.
- Parallel development of minimally 5 different plant type III PKSs by rational mutagenesis to transplant 2-PY forming activity into an evolutionarily related enzyme fold with a focus on BIS as a starting point.
- Enhancement of BIS's ability to utilize salicoyl-CoA (2-hydroxybenzoyl-CoA) as substrate. When salicoyl-CoA is used as substrate the reaction produces 4-hydroxycoumarin, a pyrone-type compound.
- Completion of 2-PS surface Cys engineering and active site engineering for final delivery of optimized 2-PS to Thrust 2.
- Initiation of starter molecule engineering in type III PKSs to begin introduction of carboxyl-bearing starter molecules to create bifunctional polyketide products readily overproduced in yeast and efficient catalysts for target molecules for Thrust 3.
- Full implementation of Minimal Models of Multidimensional Computations applied to current and developing multidimensional quantitative biochemical data sets for type III PKSs to specifically identify sets of synergistic residues contributing to enzyme stability, turnover, catalytic efficiency and reactant specificity.
- Development of an efficient orcinol synthase (OS) from the clone obtained thus far (Noel-Pichersky) that employs the same substrates as 2-PS but terminates its iterative reaction through a

ring closing Aldol reaction instead of the carbon-oxygen forming lactonization reaction of 2-PS.

Expected Milestones and Deliverables

- KASIII enzymes with modified substrate specificities that can make carboxylic acids with different chain functional groups, which will have applications as biorenewable chemicals.
- *B. subtilis* KASIII deletion mutant-based genetic screen for high-throughput screening of KASIII enzymes.
- High-throughput spectrophotometric screen for identifying KASIII enzymes with unusual substrate specificities.
- Phylogenetic trees for all five KS families.
- 2-PSs, derived from either mutagenesis of authentic *Gerbera hybrid* 2-PS or the appropriate type III PKS mutants, capable of supporting high yields of pyrone test bed products.
- Kinetic characterization of orcinol synthase (OS), its x-ray structure determination and its stability assesment to expand our polyketide test beds based upon type III PKSs and the simple substrates, acetyl-CoA and malonyl-CoA.
- Dissection of residue differences between OS, 2-PS and bibenzyl synthase (BBS) that dictate Claisen, lactonization and aldol chain-terminating chemistry to offload products.
- Interconversion of OS, 2-PS and BBS activities by site-directed multi-site mutagenesis.
- Refinement of an entirely new model to extract the most information out of systems exemplified by limited knowledge of the correlations between inputs (mutant positions) and outputs (biochemical activities) by maximizing noise entropy constrained by the input and output moments.

Member Company Benefits

Milestones and deliverables obtained as part of this project should provide small molecule end products including pyrones, short chain fatty acids, and downstream orcinols, that are integral test beds for Thrust 3. Moreover, the KAS III enzymes will provide metabolic intermediates integral to the aims of all projects associated with Thrust 1.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T1.2 – Acetoacetyl-COA -** Use of *Escherichia coli* for the production of molecules functionalized for chemical synthesis.

Thrust: Research Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Thomas A Bobik	**2/14/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Thomas A Bobik**,** Iowa State University
Graduate student: Alexandra Volker, Iowa State University
Other Personnel: Christian Bartholomay - Assistant Scientist II, Iowa State University

Statement of Project Goals

A major project goal is to genetically engineer *Escherichia coli* to produce short-chain carboxylic acids, such as those shown in figure 1, by glucose fermentation. Work will focus on production of medium chain-length (C6-C8) carboxylic acids and hydroxy-carboxylic acids (bifunctional compounds). The efficient production of medium chain carboxylic acids from coenzyme A intermediates has not been achieved. Development of an organism that produces high levels of individual carboxylic acids will be an important advance. In addition, as new catalysts become available, it will be possible to further diversify molecules shown in figure 1 to their corresponding alkanes, alkenes, α-olefins and methylketones.

A second goal of the project is to develop catalytic systems that allow the production molecules more oxidized than glucose. This requires a means for eliminating excess electrons. In general, others researchers have accomplished this by using aerobic systems where electrons are consumed by the reduction of oxygen to water. In one case, an aerobic system has been modified to minimize carbon loss through the TCA cycle (1). We will use similar approaches, and also develop an alternative anaerobic system that eliminates excess electrons by co-production of hydrogen gas. This approach that has some potential advantages. H_2 has a number of industrial uses and the introduction of oxygen into fermentation systems requires a large energy input; hence hydrogen co-production will provide a valuable co-product and may reduce process energy costs. To develop such systems, we will initially co-produce hydrogen and acetaldehyde from glucose. Both compounds have commercial application in the synthesis of industrial chemicals and acetaldehyde is produced from acetyl-CoA which the precursor of all the chemicals in figure 1. Thus, the systems developed for co-production of hydrogen with acetaldehyde will have potential application to production of all the relatively oxidized molecules in figure 1.

A third goal of the project is engineer *E. coli* such that a single carboxylic acid is produced with high efficiency. Our approach will be to identify (or engineer) enzymes with high substrate specificity. Initial studies will focus on enzymes used for CoA removal primarily thioesterases and secondarily acyl-CoA reductases, CoA transferases, and methylketone synthases.

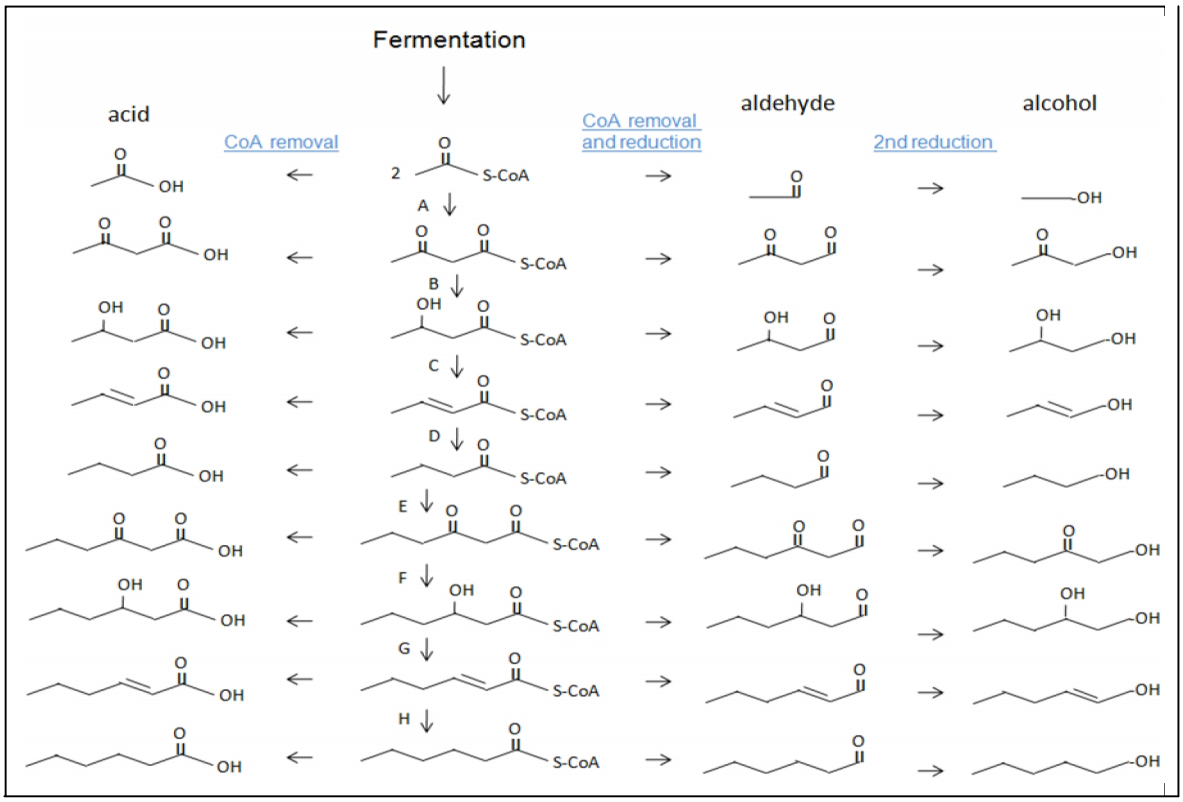

Figure 1. Pathways for the production of renewable chemicals from glucose in *E. coli*.

Project's Role in Center's Strategic Plan

The catalysts used to produce the molecules shown in figure 1 will be used by thrust 2 to develop strains of *E. coli* that efficiently produce large amounts of these chemicals. In turn, these compounds will be used in thrust 3 as substrates for chemical catalyst systems that allow the synthesis of additional classes of industrial chemicals. For example, thrust 3 has developed methods for the decarboxylation of organic acids (such as hexanoic acid) to alpha-olefins. Thrust 3 will also develop methods for the production of important polymers from bifunctional molecules such as 3-hydroxyhexanoic acid which also is a target of this project.

Fundamental Barriers and Methodologies

Other groups have produced 1-butanol and 3-hydroxybutyrate by the pathway shown in figure 1. In these systems, the crotonyl-CoA reductases have been problematic. Many are integral membrane proteins that couple to electron transport flavoproteins EftA and EftB and have low activity in *E. coli*. Hence, this enzymatic step is thought to limit 1-butanol production. This same problem is likely to apply to the production of C6 compounds by the pathway of Figure 1. To eliminate this problem, we will use NADH-dependent enoyl-CoA reductase from *Euglena*. Other groups recently reported the efficacy of this method. We have cloned and expressed this enzyme which has high activity in *E. coli*. A second problem in 1-butanol production is its toxicity. However, butanol is not a target compound for this project. The toxicity of our potential targets will be investigated by thrust 2 as they work toward high-level production and will be ameliorated by strain optimization. Three research groups have worked on the production of R- and S-3-hydroxybutyrate as starter molecule for chiral synthesis. Thus far, productivity in these systems is low but encouraging. The main barriers for this system have not yet been clearly identified but are most likely inefficiencies due to the use of a non-optimized aerobic process. Co-production of hydrogen and/or modification of aerobic metabolism will be needed to improve these processes and we are working on both of these approaches.

Additional problems in metabolic pathway engineering are imbalances in the expression of genes in an engineered pathway may lead to bottlenecks and metabolite/cofactor imbalances that inhibit growth of the producer organism and/or product formation. Balancing gene expression will be done in an iterative fashion based on analysis of product profiles. Improved flux will also be addressed by developing screens for improved production following genetic modification as well as metabolic flux analysis and omics approaches. However, these tasks are mainly the responsibility of Thrust 2.

Other general barriers to this project include (i) the production of active heterologous enzymes with the proper substrate specificity in *E. coli (ii)* the identification of currently unknown catalysts and (iii) maintenance of proper redox balance without the production of undesired co-products. Expression of heterologous enzyme requires a number of considerations including RNA stability, protein solubility, protein toxicity and post-translational regulation. Problems associated with production of active enzymes will be addressed by using gene synthesis in conjunction with computer programs that optimize codon bias, address RNA folding and stability. Development of the needed catalyst specificity will require biochemical and in vivo characterization followed by catalyst evolution if necessary. The main consideration for the identification of new catalysts is the development of efficient high-throughput screening methods and the development of appropriate screens.

Achievements

E. coli has been engineered to produce significant amounts of butyrate, hexanoate and octanoate (3.7, 0.1 and 0.03 g/L, respectively) by fermentation of glucose. We cloned, expressed and purified enzymes that convert acetyl-CoA to butyryl-CoA (reactions A-D in figure 1). These include acetoacetyl-CoA synthase, acetoacetyl-CoA reductase and crotonase from *Clostridium* as well as crotonyl-CoA reductase (CCR) was from *Euglena*. The Euglena CCR is an NADH-dependent enzyme and is expected to give better results than enzymes that use EtfAB as electron acceptor. The purified enzymes all had turnover numbers ≥ 73 sec^{-1}. These activities are suitable for a commercial process where turnover numbers of about 5-10 sec^{-1} are the minimal requirement. We also constructed a synthetic butyrate operon that includes enzymes A-D in figure 1. We have shown that this operon produces enzymes A-D in an active soluble form in *E. coli*. All four enzymes have an activity >1.6 μmole/min/mg in crude extracts. This corresponds to a maximum theoretical rate of 16 g/L/h butyrate formation under industrial conditions (2-4 g/L/h is a good target). We introduced the synthetic butyrate operon into an *E. coli* strain that has all the native fermentation pathways eliminated by genetic deletion including *adhE*, *ldhA*, *pta-ack* and *frdBC* mutants. This mutant metabolizes glucose to acetyl-CoA and formate with the latter compound being converted to $H_2 + CO_2$. Due to the presence of the synthetic butyrate operon, acetyl-CoA is converted to short chain carboxylic acids. The mutant containing empty vector and vector plus the synthetic butyrate operon was grown glucose anaerobically and fermentation products were measured by HPLC and their identity confirmed by GC-MS. The strain containing the synthetic operon initially produced about 0.3 g/L butyrate and 0.008 g/L hexanoate. After some optimization it produced about 3.7 g/L butyrate, 0.1g /L hexanaoate and 0.03 g/L octanoate, about a 12-fold increase in the case of butyrate. In contrast, the strain with the empty vector did not produce detectable amounts butyrate, hexanoate or octanoate. During the production of butyrate and hexanoate about 5.4 g/L glucose was consumed. Thus, butyrate was produced and about 80% theoretical yield. Further work will focus on improving glucose consumption (about 25% of the added glucose was utilized).

In other studies, we engineered *E. coli* to co-produce high amounts of acetaldehyde and hydrogen in good yield. The specific acetaldehyde production rate of 0.68 ± 0.20 g h^{-1} g^{-1} dry cell weight and at 86% of the maximum theoretical yield. This specific production rate is the highest reported thus far and is promising for industrial application. For these studies, we cloned and purified acetaldehyde dehydrogenase from *Salmonella*. Its turnover number was 16 sec^{-1}. We then cloned and produced acetaldehyde dehydrogenase in wild-type *E. coli* and a strain that has the native fermentation pathways eliminated by genetic deletion

including *adhE*, *ldhA*, *pta-ackA* and *frdC*. When these strains are growing anaerobically, glucose is converted to pyruvate which is split to acetyl-CoA and formate with the latter compound being converted to $H_2 + CO_2$. Subsequently, acetyl-CoA was converted to acetaldehyde by the acetaldehyde dehydrogenase we introduced by genetic engineering. In initial analyses of the quintuple mutant growing on glucose anaerobically, about 50 μmole of acetaldehyde is produced from 120 μmole of glucose. However, under the conditions used formate accumulated and hydrogen was not produced. This strain also produced significant amounts of ethanol even though the AdhE enzyme was eliminated by genetic deletion. Further studies indicated that *E. coli* produces an alcohol dehydrogenase that uses acetaldehyde (rather than acetyl-CoA) as a substrate. An allyl alcohol selection was used to eliminate most of this Adh activity and acetaldehyde production was increased. We improved the conversion of formate to $H_2 + CO_2$ by dropping the pH of the growth medium to 6.0. Prior work by others had shown that the formate hydrogen lyase of *E. coli* is activated by lower pH values. Under optimal conditions, strain ZH136 converted glucose to acetaldehyde and hydrogen in a 1:1 ratio with a specific acetaldehyde production rate of 0.68 ± 0.20 g h^{-1} g^{-1} dry cell weight, and in 86% of theoretical yield. These results are encouraging that H_2 co-production will be useful for elimination of unwanted electrons during the production of molecules more oxidized than glucose. In addition, other CBiRC personnel are conducting feasibility studies on upgrading acetaldehyde to longer chain organic chemicals.

Recently, we began work on the use of thioesterases for increasing the yield and specificity of short chain carboxylic acid production by fermentation. The synthetic operon which was expressed in *E. coli* (produces enzymes A-D in figure 1) did not include enzymes for removal of CoA from butyryl-CoA. We presumed this would occur via endogenous thioesterase enzymes and this was observed to a reasonable extent. However, the endogenous enzymes for CoA removal could be rate limiting for carboxylic acid production. To address this we cloned several thioesterase genes from *E. coli*. In the future, we plan to express these genes and look for increased product formation. We have also genetically deleted 5 thioesterase genes from the chromosome of *E. coli*. We plan to asses the effects of these deletions on product formation later in 2013.

We have also begun the genetic engineering of *E. coli* for the production of additional classes of carboxylic acids. We cloned genes for the production of propionyl-CoA. This was done to allow the formation of odd-chain length carboxylic acids. The synthetic operon we constructed for the production of butyrate is expected to mediate the condensation of propionyl-CoA with acetyl-CoA to form a C5 compound that is converted to pentanoic acid. In initial studies we have produced small of pentanoic acid. Further work will be aimed at improving yields.

We have also made clones that we expect will allow the production of omega hydroxyl carboxylic acids when combined with the synthetic butyrate operon described above. We are currently testing enzyme expression by these clones and plan to test their usefulness in future studies.

Other Relevant Work

To our knowledge, no other group has used a CoA-based pathway to efficiently produced medium chain-length carboxylic or omega hydroxy carboxylic acids in *E. coli*. A number of other researchers have utilized heterologous expression of one or more of the enzymes leading from acetyl-CoA to butyryl-CoA for the production of 1-butanol or isopropanol in *E. coli* (2-6). Several additional papers describe the engineering of pathways to produce 3-hydroxybutyrate (7, 8). Our research parallels these previous studies as far as the production of butyryl-CoA, but we plan to extend the butyrate pathway to produce a range of medium chain-length carboxylic and omega hydroxy carboxylic acids. Nonetheless, prior studies on the production of C4 compounds via CoA derivatives provides information relevant to this project. Studies by Inui et al. (5)

suggested that the lack of appropriate electron transfer proteins (EtfAB) impaired flux through from acetoacetyl-CoA to 1-butanol. EtfAB are needed for the activity of crotonyl-CoA reductase (CCR). We plan to use CCR from *Euglena* which uses NAD^+/NADH as a co-substrate rather than EtfAB. In other studies, Vadali et al. demonstrated that genetic modification *E. coli* (deletions, modifications) as well as manipulation of cofactor levels could be used to redirect acetyl-CoA into specific pathways to attain desired end-products (9, 10). Hanai, et al. successfully used the acetoacetyl-CoA pathway to produce isopropanol in *E. coli* in titers greater than that of native producers by using codon optimized synthesized genes from two *Clostridia* species and *Thermanaerobacter brockii* (4). Others expanded this line of research with a modified strain for isopropanol synthesis that produced 227 mM isopropanol, and a sixth the amount of acetate compared to wild type thus demonstrating the ability to significantly target synthesis of a desired molecule with a concomitant shift of flux away from a competing allosteric regulator (6). Collaboration with investigators in Thrust 2 to define metabolic fluxes involved in the production of targeted CoA intermediates will be necessary. In general, the scientific literature has shown *E. coli* metabolism to be highly malleable in regards to redox state and flux manipulations making it amenable to the introduction of heterologous pathways for green chemical production (5, 6, 11, 12). Hence, our goal to express non-native enzymes in *E. coli* to produce 4- and 6-carbon CoA intermediates from glucose for downstream modifications by Thrust 3 has a solid basis in the literature.

Plans for the Next Year

We will work on further increasing the yields of various carboxylic acids by *E. coli* fermentation. This will be done by tuning gene expression levels and by engineering in additional enzymes needed to maintain redox balance. Specifically, we will introduce a NAD-dependent formate dehydrogenase to provide the additional NADH that is required for the production of carboxylic acids greater that C4.

We will also work on improving the specificity of product production. Currently we are producing mixtures of butyric, hexanoic and octanoic acids. We will try to produce just one compound in higher yields. Toward this goal we have genetically deleted 5 native thioestease genes found in *E. coli*. Fermentation studies will be used to test the effects of these mutations on carboxylic acid production. These studies will work help define the endogenous systems that mediate the conversion of CoA derivatives to their corresponding carboxylic acids + CoA. Once these systems are defined it will be possible to replace them with alternative more specific systems to allow the production of particular carboxylic acids as products.

We will continue work on the production of odd-chain length carboxylic acids by engineering systems that produce propionyl-CoA into *E. coli*. Initial studies have shown that propionyl-CoA is serves as a substrate for enzyme A-D figure 1 such that valeric acid is produced. We will work on optimizing this process and using a similar approach to produce longer odd-chain-length carboxylic acids. Controlling product specificity will require appropriate CoA removal enzyme as described above.

We will continue studies on enzyme systems for the production of omega hydroxy acids. These studies are currently in the early stages. We have cloned some of the needed genes are testing for production of active enzyme. Once we have verified that our clones produce active enzyme, fermentation studies will be conducted to identify possible new products.

Expected Milestones and Deliverables

We expect to produce the following compounds.

1. valeric acid

2. 3-hydroxyvaleric acid

3. heptanoic acid
4. 3-hydroxyheptanoic acid

Member Company Benefits

The proposed task will provide microbial catalysts for the production industrial chemicals or platform chemicals.

Commercialization / Technology Transfer

This will be done by consulting with member companies. There has been no technology transfer to date.

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NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T1.3 Acetyl-CoA/Propionyl-CoA Synthetase -** Biocatalysts for Diversifying Precursor Pools for FAS/PKS Synthesis

Thrust: Research Thrust 1 - New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Basil J. Nikolau	**02/14/2013**	**03/01/2012 to 02/28/2013**

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Undergraduate Student: Taylor J. Edgar, Iowa State University
Undergraduate Student: Chelsea L. Sholander, Iowa State University

Statement of Project Goals

The specific goal of this project is to develop biocatalysts (enzymes and enzyme systems) that can provide novel acyl-CoA precursors for the fatty acid synthase (FAS)/polyketide synthesis (PKS) system. In most biological systems, products generated by FAS/PKS are straight chain and of chain lengths of even number carbon atoms (e.g. 8,10, 12, etc.). This is the result of the fact that acetate is used to both prime and extend the acyl chain. The primers for these FAS/PKS systems are introduced into the biosynthetic machinery as acyl-CoAs. In order to diversify the range of products that can be generated by FAS/PKS systems, this project seeks to discover and/or bioengineer acyl-CoA synthetases that can produce acyl-CoA primers that are odd numbered (i.e., propionyl-CoA) or branched chain (i.e., isobutyryl-CoA, isovaleryl-CoA or 2-methylbutyryl-CoA) primers. The incorporation of such primers into the FAS/PKS biosynthetic machinery would diversify the products of FAS/PKS by enabling the biosynthesis of odd-numbered straight-chain carboxylic acids, iso-branched odd and even numbered carboxylic acids, or anteiso-branched odd and even numbered carboxylic acids. The immediate goal of the project is to provide well characterized, high activity enzymes that can generate acetyl-CoA, propionyl-CoA, isobutyryl-CoA, isovaleryl-CoA or 2-methylvaleryl-CoA for Thrust II.

Project's Role in Center's Strategic Plan

The objective of CBiRC is to create new biologically-derived platform chemicals to replace existing petroleum-derived chemicals for the synthesis of commodity chemicals. This will be accomplished by creating a new series of biological precursors modified from intermediates of polyketide/fatty acid synthesis that can then be converted by chemical processes into feedstock compounds. The biochemical catalysts that will be created by Thrust 1 will be designed to mimic existing PKS/FAS systems but altered to create and release a variety of small reaction intermediates

instead of the long chain fatty acids that the systems currently produce. In order to accomplish this goal we will need to identify or engineer enzymes that can create novel acyl-CoA molecules that can serve as precursors for the systems, modified ketoacyl-synthases that can use these novel substrates and new thioesterases and methylketone synthases that can release the desired intermediates. The purpose of this project is to develop the acyl-CoA synthetases that can provide novel acyl-CoA primer molecules as substrates for the process.

This project will begin by developing a modified acetyl-CoA synthetase that can be used for several purposes. It will allow us to develop enzymology capabilities needed to work with this family of proteins. It will also provide a reagent for Thrust II that will allow them to modify *E. coli* to increase its capacity to use acetate as a substrate. This will be an important organism for the Center's long-term vision in that it will allow for the experimental modification of the rate of acetyl-CoA production and thus evaluate the effect of altering metabolite flux in the middle of the pathway. Our longer term goals are to develop acyl-CoA synthetases that will provide propionyl-CoA and branched chain CoAs as precursors.

Fundamental Barriers and Methodologies

In order for this project to be successful two sets of initial goals need to be accomplished. First, three different acyl-CoA synthetases, specific for acetate, propionate, and isobutyrate need to be discovered (or bioengineered) and characterized. These would provide the key biocatalysts for the project. Second, once these enzymes are available, they will need to be modified to provide maximum activity when expressed in *E. coli* or yeast. Studies in this and other laboratories have shown that these enzymes are controlled by two independent post-translational systems, an oxidation of enzyme thiol groups and an acylation of the enzyme active site. We will need to understand the biochemistry of these mechanisms and to create mutants that are not regulated in order to achieve maximum expression.

Achievements

from 3/1/2012 to 2/11/2013

Circular Dichroism of Acetyl-CoA Synthetase Homologs and Arabidopsis Acetyl-CoA Synthetase Mutants

Circular dichroism is being used to investigate differences in folding between acyl-CoA synthetases with different substrate specificities as well as the effects of point mutations on the overall fold of the Arabidopsis ACS. Spectra are generated by a Jasco J-700 spectropolarimeter and are capable of providing estimations of the α-helix, β-sheet, and random coil content in a folded protein. Figure 1 shows example spectra, and Table 1 shows the quantified data extracted from those spectra.

Figure 1: Circular dichroism spectra of Arabidopsis ACS (aACS), *E. coli* ACS (eACS), *E. coli* PCS (ePCS), and *Pseudomonas chlororaphis* ICS (pICS)

Data collected from circular dichroism can be analyzed by JFIT to calculate estimated percentage of α-helix, β-sheet, and random coil. Using these numbers we can compare folds quantitatively. These data show that the homologous proteins all have similar folds with the exception of *E. coli* ACS which shows higher α-helix content then the other proteins.

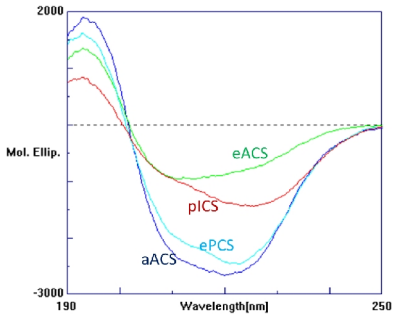


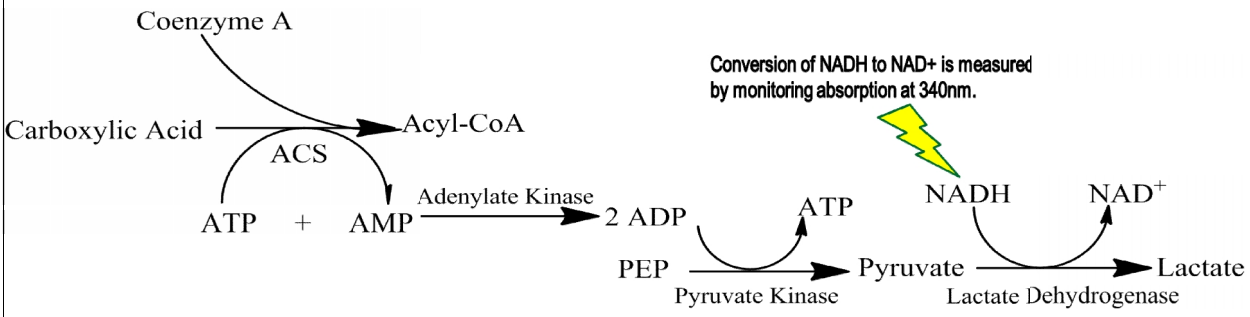
Table 1: Comparison of folds between aACS, eACS, ePCS, and pICS show similar folds in all but *E. coli* ACS

Enzyme	α-helix	β-sheet	Random Coil
aACS	9.88	53.47	36.64
eACS	26.32	45.52	28.16
ePCS	9.46	52.01	38.53
pICS	9.91	50.11	39.98

Development of Substrate-Dependent Colorimetric Assay for Acyl-CoA Synthetase activity

In order to further analyze and confirm the enzymatic rates of the Arabidopsis ACS WT and mutated forms, along with investigating the rates and substrate specificities of the bacterial acyl-CoA synthetases, we have developed a colorimetric plate based assay for determining substrate specific activities. Using this method we can analyze as many as 96 samples at a time irrespective of the enzyme or substrate.

Figure 2: Schematic representation of reactions in colorimetric assay. The acyl-CoA synthetase (ACS) is coupled to three other reactions in order to monitor activity.



This new method utilizes three coupled enzymes in conjunction with our enzyme of interest to cause a decrease in NADH levels when the enzyme is active. All the acyl-CoA synthetases we are

studying utilize an ATP, which is hydrolyzed to form AMP during the reaction to form an acyl-CoA. The ATP and AMP are utilized by an Adenylate Kinase enzyme to form 2 ADP molecules. ATP is formed from the ADP when Pyruvate Kinase forms pyruvate from phosphoenolpyruvate. Pyruvate is converted to lactate by Lactate Dehydrogenase which oxidizes NADH to NAD^+, producing a decrease in absorption at 340nm which is measured to determine enzymatic activity. Using a multi-well plate format multiple conditions can be assayed at one time and kinetic data can be generated from the rate of decrease in absorption at 340nm.

Other Relevant Work

A manuscript describing the dithiol regulation of acyl-CoA synthetases has been accepted for publication. While other groups have worked on regulation by acylation, no one has published a mechanism for overcoming this feedback inhibition.

Plans for the Next Year

- Confirm Arabidopsis ACS mutant activities on different substrates using a colorimetric assay.
- Compare activities of mutant ACS enzymes to those of *E. coli* ACS, *E. coli* PCS, and *P. chlororaphis* ICS using colorimetric assay.
- Generate and characterize $Ile^{312}Ala$ and $Thr^{313}Tyr$ single ACS mutants to determine their individual effects on substrate specificity.
- Study the *in vivo* effects on acyl-CoA pools in *E. coli* expressing ACS, ACS mutants, PCS and ICS enzymes via LC-MS-MS analysis.
- Analyze fatty acid profiles from *E. coli* expressing ACS, ACS mutants, PCS and ICS enzymes via GC-MS analysis. This will access the impact of new FAS primers on fatty acid synthesis products.

Expected Milestones and Deliverables

The deliveries for this project are the genes for the modified forms of these enzymes along with the knowledge of how to manipulate and assay them. We expect these to be done within the next two years and delivered to Thrust II.

We anticipate a an additional manuscript from this project, and this will be the MSc thesis of graduate student, Jason Hart. We anticipate that these will be completed in August 2013, when Jason Hart will leave ISU to begin a PhD at another institution.

Member Company Benefits

A disclosure has already been released on the redox regulation mechanism and one of the partner companies has expressed interest in this technology. An early draft of the manuscript describing this biocatalyst has been forwarded to them. We will keep them informed as additional biocatalysts become available and as we gain information on the potential of these enzymes to modify metabolism in *E. coli*.

We anticipate a second disclosure once kinetic data from all mutant enzymes are collected, based on Jason Hart's MSc thesis/manuscript

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T1.4 – Acyl-CoA Carboxylases -** Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems

Thrust: Research Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Basil J. Nikolau	**02/14/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Basil J. Nikolau, Iowa State University
Other Faculty: Peter J. Reilly, Iowa State University
Graduate Students: Bryon Upton, Iowa State University
Graduate Students: Yingfei Chen, Iowa State University
Graduate Students: Kiran-Kumar Shivaiah, Iowa State University
Other Personnel: Marna Yandeau-Nelson, Iowa State University
Undergraduate Student: Taylor Edgar, Iowa State University

Statement of Project Goals

The goal of this project is to develop acyl-CoA carboxylases (ACCase) that can activate diverse acyl-CoA molecules to produce novel substrates for 3-ketoacyl-ACP synthases or other KS components of polyketide synthases (e.g., pyrone synthase). Normally, acetate units are activated for polyketide synthesis by carboxylating acetyl-CoA to malonyl-CoA. The loss of the CO_2 group in subsequent reactions drives the condensation reaction catalyzed by the 3-ketoacyl synthases. One of the other projects in this thrust is designed to produce branched-chained, odd-numbered acyl-ACP molecules and pyrones. In order to achieve these goals, this project seeks to enhance and modify the malonyl-CoA substrate that will be used in the biosynthesis of these products; i.e., we will need to develop acyl-CoA carboxylases with enhanced activity and altered substrate specificities.

Project's Role in Center's Strategic Plan

The goal of the thrust is to identify or bioengineer biocatalysts that can be used to produce a diverse group of biochemicals from the intermediates of fatty acid biosynthesis. While the products of this pathway are normally even-numbered and straight-chained, our intention is to also produce molecules that contain chemical functionalities at the omega-end of the product molecules (e.g., branched-chain, odd-numbered carbon-compounds, ring-structures, hydroxylated or halogenated products). In order to accomplish this, we will need to incorporate altered primers into the polyketide synthesis biocatalysts and enhance the supply of malonyl-CoA, the extender substrate of polyketide synthesis biocatalysts. This project is designed to produce biocatalysts that address the latter question, enhancing the supply of malonyl-CoA. This will be accomplished by creating modified acetyl-CoA carboxylases as specific biocatalysts. Initially, we will survey the acyl-CoA carboxylases in a range of microbial systems in order to identify enzymes with diverse substrate

specificities. Structural analyses and site-directed mutagenesis will be used to extend the natural range of substrates used and to create the necessary biocatalysts for the project that will use unusual acyl-CoAs as substrates. The Reilly group will construct a database of the acyl-CoA carboxylase genes, proteins, and structures in the literature and public databases.

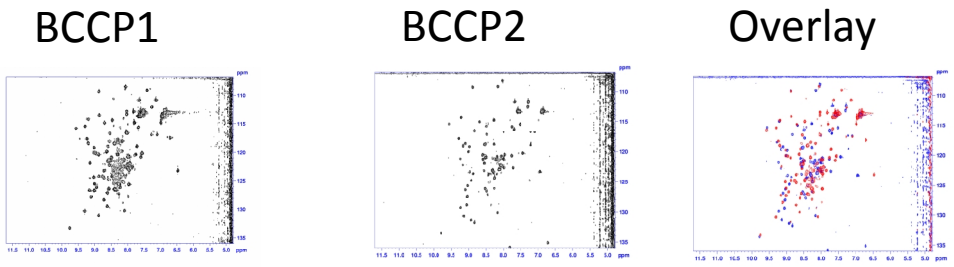
Fundamental Barriers and Methodologies

- Purification of the heteromeric ACCase from many sources is difficult due to the subunits dissociating during purification. Because there are multiple genes encoding each of the ACCase subunits [biotin carboxyl-carrier protein (BCCP), biotin carboxylase (BC), carboxyltransferase alpha (CT-alpha) and beta (CT-beta) subunits], which is a large multimeric complex, subsequent purification of ACCase result in a heterogeneous mixture of isoforms within holo-ACCase. To overcome this barrier, we have built a heterologous expression system in *E. coli* to express each ACCase subunit individually and in combination with the other subunits of ACCase.
- Previous studies indicate that over-expression of ACCase subunits in *E. coli* results in the accumulation of predominantly insoluble, inactive biocatalysts. By co-expressing subunits we have increased the solubility and functionality of the expressed ACCase.

Achievements

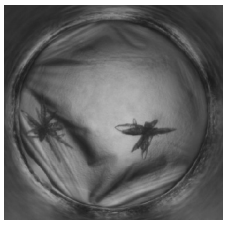
Achievements between 3/1/2012 to 1/31/2013

1. The BCCP1 and BCCP2 proteins were subjected to nuclear magnetic resonance spectroscopy. The 2-Dimensional Hetero-nuclear single quantum coherence spectroscopy was performed. Later 3-Dimensional NMR experiments were carried out to get different spectra, which were further used to predict the structure of BCCP1. Initial data shows the peaks which are part of 2-D HSQC are from the C-terminus of the BCCP1.



2. High-throughput X-ray crystallography experiments were performed on Biotin Carboxylase enzyme (HWI, New York). The optimal conditions and reagent composition required for the growth of the crystals were obtained. The same experiments have to be repeated at Iowa State University using hanging drop vapour diffusion method.

Crystals of Biotin Carboxylase: (Different conditions)



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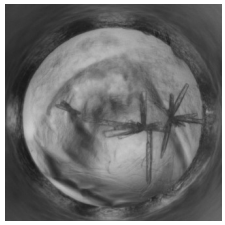
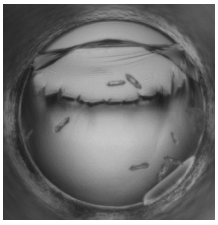
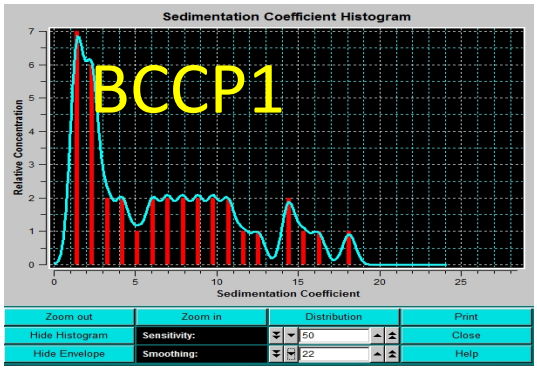
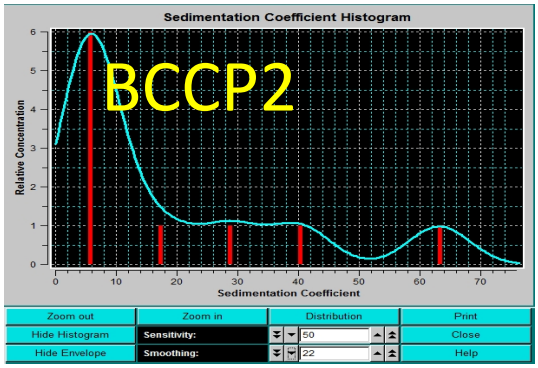


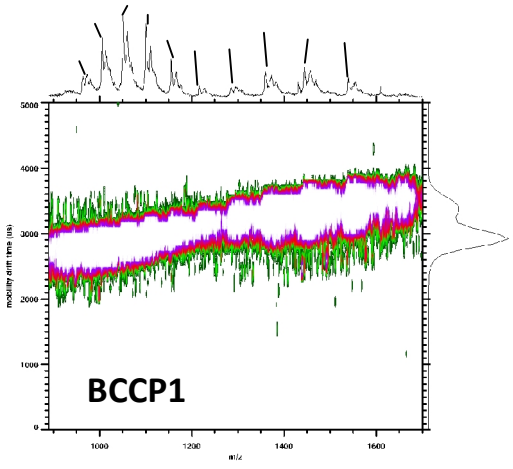


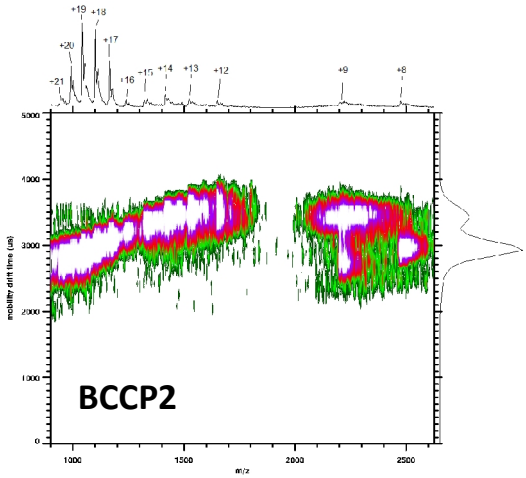
3. Analytical Ultracentrifugation indicates that both BCCP isoforms prefer a dimer but they also complex into oligomers.



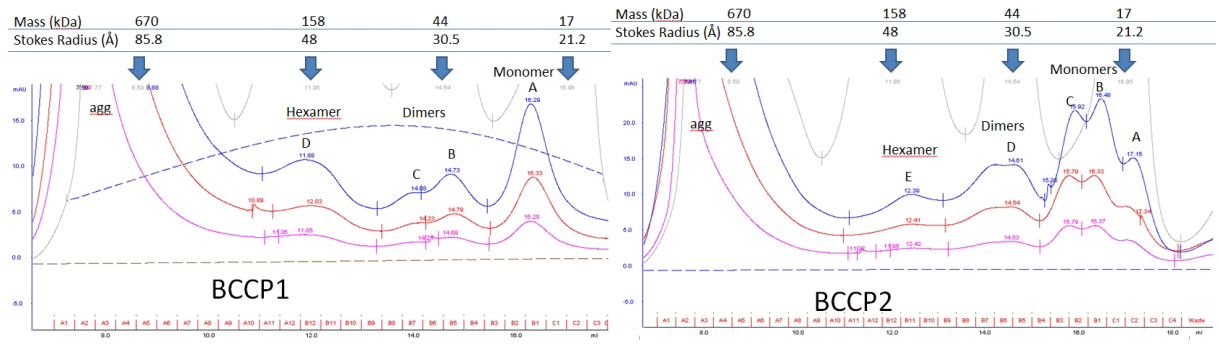


4. Ion mobility mass-spectroscopy of both BCCP isoforms across many charge states. Both BCCP1 and BCCP2 appear mainly as monomers with a slight hint of a dimer within the BCCP2 spectrum (indicated by *)





5. FPLC analysis of BCCP isoforms at 3 concentrations shows hexamer, dimer, and monomeric states. BCCP1 (left panel) appears to have two unique dimeric conformations while BCCP2 (right panel) in addition has 3 monomeric conformations



Other Relevant Work

In addition to acetyl-CoA carboxylase, 3-methylcrotonyl-CoA carboxylase (MCCase) is being studied. This enzyme catalyzes the carboxylation of 2-methylcrotonyl-CoA to form 3-methylglutaconyl-CoA and contains only two subunits, an MCCA which contains both BC and BCCP domains, and MCCB, which contains a functional CT domain. In *Arabidopsis thaliana* MCCA exists as both a long and a short isoform, generated by alternative splicing of *mcca* mRNA. Computational modeling of both MCCA isoforms suggests that both may fold into potentially active enzymes, with the difference between the two being an alpha-helix distal to the active site. It is unclear, however, whether the presence or lack of this helix has an effect on protein-protein interactions, which could affect activity. By understanding the diversity within and between ACCase and MCCase, it may be possible to understand the biochemical control of substrate specificity at the CT active site.

Plans for the Next Year

- Completion of kinetic analysis for ACCase
- Define the oligomeric status for ACCase subunits individually and in concert with combinations of subunits.
- Solution NMR (HSQC and NOESY) studies of BCCP1 and BCCP2 to solve solution structures for each isoform.
- Initial X-ray crystallography studies of BC to compare Arabidopsis BC structure to that of known bacterial structures.
- NMR-based characterization of ACCase complexes – specifically between BC and BCCP1 and BC and BCCP2.

Completed Milestones:

- Purification and characterization of ACCase complexes; completed May, 2012.
- Purification and characterization of MCCase complexes; completed December, 2012.
- Phylogenetic analysis for all acyl-CoA carboxylase families; completed October, 2012.
- Based on phylogenetic analyses of ACCase subunits from diverse organisms, synthesis and expression of potentially novel acyl-CoA carboxylase enzymes; completed October, 2012.

Expected Milestones and Deliverables

- Solution NMR studies of BCCP1 and BCCP2 isoforms and their interactions with BC and CT components – Anticipate delivery, December 2013
- Kinetic analysis of the ACCase two half-reactions (BCase and CTase) with BCCP1 or BCCP2 isoforms – Anticipate delivery, September, 2013
- Solution structure and X-ray structure determination of ACCase components (BCCP isoforms and BC) – Anticipated completion is difficult to predict as these are dependent on unknown behaviors of proteins in crystallization procedures, but a "go/no-go" decision will be made by Dec 2013.

Member Company Benefits

This project will generate novel biocatalysts that can generate "elongating substrates" for fatty acid synthases and/or polyketide synthases. Depending on the novel substrate that will be generated, the use of these biocatalysts will result in the incorporation of internal methyl- or ethyl-branches in the resulting alkyl chain. Another potential benefit of this project derives from the fact that the acyl-CoA carboxylase biocatalyst is considered to be an important regulatory reaction of fatty acid synthesis; thus, this research has potential to enhance the production of fatty acids. These are questions that many of CBiRC's industrial partners would like to address, in order to enhance their biorenewable chemical platforms based upon fatty acid biosynthesis.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T1.5 – Methylketone Synthase/Thioesterase -** Development of Methylketone Synthase Enzyme Adapted for Production of Short-Chain Methylketones

Thrust: Research Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Eran Pichersky	**02/26/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Dr. Eran Pichersky, Dr. Thuong Nguyen, Mr. Geng Yu – University of Michigan

Dr. Joseph Noel, Dr. Yongxia Gao – Salk Institute

Statement of Project Goals

Tomato methylketone synthase1 (MKS1) and MKS2 convert intermediates in the fatty acid biosynthesis pathway, namely 3-ketoacyl-ACP, to mathylktones. In tomato, MKS2 "grabs" the C12, C14 and C16 3-ketoacyl-ACPs and hydrolzyes the ACP. MKS1 then decaroxylates the resulting products to give C11, C13 and C15 methylketones. The overall aim of this project is to use these two enzymes to terminate fatty acids at an earlier cycle in the chain elongation process, to provide methylketones of shorter chain length such as C5 and C7, and to achieve a high level of production of such methylketones. To achieve the synthesis of short chain methylketones, we will use two approaches: Engineering the existing tomato MKS1 and MKS2 by *in vitro* mutagenesis based on information derived from the crystal structure of the protein (this work will be done with Dr. Noel's lab at the Salk) and by looking for additional natural variants of MKS1 and 2 (which will subsequently be structurally characterized by Noel's group).

Project's Role in Center's Strategic Plan

Providing plant genes for enzymes that produce short methylketones.

Fundamental Barriers and Methodologies

Cloning genes from plants, expression in *E. coli*, testing activities *in vitro* using in-house synthesized substrates.

Achievements

Goal 1: Develop an MKS2 that catalyzes the formation of short-chain methylketones

Task 1: Perform *in vitro* mutagenesis of tomato MKS2

Progress: The lack of structural information on MKS2 is slowing down our work on *in vitro* mutagenesis. We have obtained several mutants so far based on some structural predictions

(from comparisons with other related proteins), but so far we have not obtained mutants that better hydrolyze shorter precursors.

Task 2: Clone and characterize new MKS2 natural variants from various tomato accessions and from other species, express them in *E. coli* and characterize their activity
MKS2 proteins are widely distributed in the plant kingdom and they can produce methylketones when expressed in *E. coli*. We had previously analyzed MKS2 from two tomato species and from Arabidopsis (Arabidopsis has three functional MKS2 genes). We expressed these genes with varying success in *E. coli* and showed their activity by measuring methylketone production (by GC-MS). We have observed the production of methylketones from the MKS2 cDNAs of the two different tomato species and from two of the three Arabidopsis MKS2 cDNAs. Promisingly, each MKS2 produces a somewhat different range of methylketones. One tomato MKS2 and one Arabidopsis MKS2 produce predominantly C7 and C9 methylketones, and less of the longer ones. We also identified MKS2 from additional plant species, including momocots (rice, corn) and a gymnosperm (Sitka spruce), and they too produce methylketones when expressed in *E. coli*. The two tomato MKS2 cDNAs have been sent to Thrust 2 group members for optimization for microbial expression. (paper published: Park J, María Rodríguez-Moyá, Li M, Pichersky E, San K-Y, Gonzalez. Synthesis of methyl ketones by metabolically engineered *Escherichia coli*. Journal of Industrial Microbiology & Biotechnology 39:1703-1712 (2012). We also purified these proteins and did *in vitro* enzymatic assays. This year we have concentrated on the plant rue (*Ruta graveolens*), which is known to produce mostly 2-nonanone. We have obtained several cDNAs encoding MKS2 from this plant, and are in the process of testing the encoded proteins for activity.
In addition, we have also tested the production of methylketones in transgenic plants, as substitutes for microorganisms, simply to see if smaller methylketones could be produced. We expressed the tomato MKS1 and MKS2 in transgenic Arabidopsis plants. The seedlings were shown to produce methylketones at low levels, but mostly tridecanone. Interestingly, the fatty acid profiling of the seedlings detected low concentrations of free lauric and myristic acids

Task 3: Work with Noel's group to structurally characterize MKS2 enzymes
Progress: All the MKS2 cDNAs described above have been sent to Dr. Noel's lab and they have begun the structural work. In addition, we are working with the Noel's group to develop MKS1-MKS2 fusion proteins that will have higher levels of activity and will also be more amenable to structural investigations. Thus far we have obtained atomic resolution structures of several MKS1 orthologs from cultivated tomato (*S. lycopersicum* – see below), verified that they catalyze decarboxylation of beta-ketoacids and have identified by protein x-ray crystallography, divalent metal-binding to one member of this SlMKS1 family that contribute to decarboxylation.

Goal 2: Improve the decarboxylase activity of MKS1 with shorter 3-ketoacids

Task 1: Perform *in vitro* mutagenesis of tomato MKS1
Progress: Together with the Noel group, we have now obtained several MKS1 mutant proteins which are much more active with shorter 3-ketoacids (e.g., C7) than with C14 and have verified and extended the structural analysis of these mutants at near atomic resolution.

Task 2: Clone and characterize new MKS1 natural variants from various tomato accessions, **express them in *E. coli* and characterize their activity**

Progress: Plants outside the tomato genus Solanum do not have proteins that are closely related to MKS1. However, the cultivated tomato (*S. lycopersicum*) has at least three MKS1 homologs, and we are now studying then in some detail. Some have already been observed to be more active with short 3-ketoacids, but we need to further characterize them.

Task 3: Work with thrust 2 to analyze flux in *E. coli*

Progress: The tomato MKS1 cDNA, two tomato MKS2 cDNAs and the Arabidopsis MKS2 cDNAs have already been send to Thrust 2 investigators and some have already been expressed, with the results that methylketones have been produced.

Other Relevant Work

Plans for the Next Year

Analysis of rue MKS2 for substrate and product specificity and 3D analysis.

Expected Milestones and Deliverables

Several enzymes that can synthesize a range of short-chain methylketones.

Member Company Benefits

A patent application for the plant enzyme Methylketone Synthase 2, a thioesterase that hydrolyzes 2-ketoacyls, has been filed. This enzyme will be very valuable for producing short methylketones in bacteria and plants. A two-year license was obtained by Monsanto in 2011 for evaluation of the potential of this gene in plant defense.

8NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T1.6 – Thioesterases -** Characterization of Novel Biocatalysts (Thioesterases) for Diversifying FAS/PKS Metabolic Pathways

Thrust: Research Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date (in U.S. date format):	Reporting Period:
Basil J. Nikolau	**02/17/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Basil J. Nikolau, Iowa State University
Other Faculty: Peter J. Reilly, Iowa State University
Graduate Students: David Cantu, Iowa State University
Graduate Students: Yingfei Chen, Iowa State University
Graduate Students: Fuyuan Jing, Iowa State University
Undergraduates: Jarmila Tvaruzkova, Iowa State University (Charles University, Prague)
Undergraduates: Sam Condon, Iowa State University
Undergraduates: Colin Hueser, Iowa State University
Undergraduates: Sara Pederson, Iowa State University
Other Personnel: Marna Yandeau-Nelson, Iowa State University

Statement of Project Goals

The goal of this project is to identify and characterize novel biocatalysts from plant and microbial polyketide synthase (PKS) systems for the purpose of diversifying the fatty acid synthase (FAS) systems of *E. coli* and the yeast *Saccharomyces cerevisiae*. This project specifically targets enzymes that could be used to prematurely terminate FAS at shorter chain lengths than normal. Specifically, we have targeted acyl-ACP thioesterases (EC 3.1.2.14 and EC 3.1.2.21) as the biocatalysts that will prematurely terminate FAS, and acyl-CoA thioesterases (EC 3.1.2.2 and EC 3.1.2.20) as the biocatalysts that can terminate CoA-dependent acyl ester biosynthetic pathways. Initial goals aim to clone and express orthologs of these biocatalysts from diverse biological sources that are known to show distinct substrate specificities. These proteins will be characterized in order to determine the structure-function relationship to construct biocatalysts with increased catalytic efficiency and altered substrate specificity for shorter fatty acids. In parallel, we will construct databases of all the thioesterase genes, proteins, and structures uncovered in the literature and public database sources.

Project's Role in Center's Strategic Plan

One of the major goals of the Center is to create a biological system based on FAS/PKS, which can produce a suite of chemicals that are shorter than 6- or 8-carbon atoms. One means for achieving this goal is to find biocatalyst(s) for stopping the elongation process of FAS at less than 8-carbon atoms. Normally, FAS in *E. coli* and yeast is terminated at 16 and 18 carbon atoms. However, plant systems exist that can terminate the elongation process of FAS with different versions of acyl-ACP

thioesterases that have specificity for chain lengths of 8, 10, 12, and 14 carbon atoms. The goal of this project is two-fold: 1) Find and characterize the molecular details of the nature of these thioesterases that display different substrate specificities; and 2) based on the understanding of the design-principle of these thioesterases, create by mutagenesis thioesterases that have the desired substrate specificities.

Fundamental Barriers and Methodologies

To design novel biocatalysts that can prematurely terminate FAS at shorter chain lengths, the fundamental knowledge that is required is how to elucidate the structure-function relationship of this biocatalyst. However, it's not well understood how acyl-ACP thioesterases recognize different substrates due to the lack of structures. To overcome this barrier we will determine the structures of acyl-ACP thioesterases that have different substrate specificities. Briefly, several acyl-ACP thioesterases will be over-expressed, purified and used to study the structure-function relationship. Novel thioesterases can then be rationally designed based on this knowledge. A high-throughput method will also be developed to test the bioactivity of different thioesterases and their mutants.

Achievements

From 3/1/2012 to 2/29/2013:

- Three residues (Asn, His, and Cys) were previously proposed as catalytic residues of plant acyl-ACP TEs (Mayer and Shanklin, J Biol Chem, 2005). However, a multiple sequence alignment of both plant and bacterial TEs revealed that these proposed catalytic residues are not conserved among all TEs, but revealed several other residues that are universally conserved. Based on such a multiple sequence alignment and structure modeling, we proposed that Cys is not a catalytic residue, but an adjacent Glu and another Asp may be involved in the catalytic residues. Site-directed mutagenesis was performed on CvFatB2 (Cuphea viscosissima) to verify these predictions. Mutating the previously proposed active site residue Cys to Ala or Ser did not greatly impact the TE activity, whereas mutating Glu and Asp to Ala or Gln and Asn dramatically decreased the activity of TE. The wild type (CvFatB2) and mutant proteins were heterologously expressed and purified from E. coli. The CD spectra of mutant proteins were very similar to the wild type protein, suggesting that the mutation did not affect protein folding and thus the loss of activity was not caused by mis-folding but was a consequence of each point mutation. Our results prove that Cys is not a catalytic residue, but the adjacent Glu and another Asp may be catalytic residues.
- To identify residues that determine substrate specificity of acyl-ACP TEs, ~20 chimeric TEs have been generated and characterized from two plant TEs, CvFatB1 and CvFatB2, each of which show different substrate specificities. Specifically, these TEs share >70% amino acid identity, but CvFatB1 is C8/C10 specific, and CvFatB2 is C14/C16 specific. By comparing the sequences and substrate specificities of those chimeric TEs, the N-terminus "hotdog" domain was identified to affect the substrate specificity. More specifically, fragment III is the most important region that determines the substrate specificity of TE.
- Based on bioinformatic analysis, 13 residues were predicted to affect substrate specificity of TE. Site-directed mutagenesis was performed to verify these residues. Among them, 5 residues proved to affect the substrate specificity.

Other Relevant Work

Novel acyl-ACP thioesterases obtained in this research are currently being used to engineer FAS metabolic pathway in *E. coli* and yeast for producing short chain fatty acids in the Thrust 2 labs of Ka-Yiu San and Nancy DaSilva, respectively.
In a complementary effort, the Reilly group has identified and classified 16 families of acyl carrier proteins (ACPs) from sequences currently available in public databases. Normal mode analysis was conducted to compare dynamic structures and computational methods have been used to predict the three-dimensional structures of ACPs for which structures are not available. Continued work in this area will address how the acyl chain "fits" within the ACP protein structure and how TEs (and other enzymes) gain access to the acyl-chain within the acyl-ACP structure for catalysis.

Plans for the Next Year

- Using the new *in vitro* enzymatic assay, analyze the kinetic characteristics of acyl-ACP TEs to understand the catalytic mechanism.
- Perform directed evolution on acyl-ACP TEs and screen for novel TEs with GC-MS.
- Optimize crystallization conditions for fjTE10 and fjTE20; purify other acyl-ACP TE proteins (e.g. CvFatB1 and CvFatB2) for crystallization screening.
- Set-up for solving the crystal structures of several acyl-ACP TEs (e.g., fjTE10, fjTE20, CvFatB1, and CvFatB2), which have diverse substrate specificities. Understand the catalytic mechanism of TE and the structural basis that confers TE substrate specificity.
- Rationally design acyl-ACP TEs with desired activities, such as TEs specific for short chain fatty acids and TEs that produce substituted fatty acids.

Expected Milestones and Deliverables

- Identification of residues that determine the substrate specificity of acyl-ACP TE by year 2012
- Crystal structures of several acyl-ACP TEs with different substrate specificities by year 2013
- Understanding the structure-function relationship of acyl-ACP TE by year 2013
- Acyl-ACP TEs with rationally designed activities by year 2013

Member Company Benefits

This project has identified novel biocatalysts that can hydrolyze the fatty acid from acyl-ACP, terminating the fatty acid elongation process at considerably shorter chain lengths than normal, specifically at 4-, 6-, 8-, 10 and 12- carbon chain lengths. In addition, novel TEs that can produce unsaturated FAs or methylketones have also been identified. These novel enzymes have benefits for companies that have products in the detergent and surfactant markets, and with bioenergy companies. We have established collaborations with two of our partner companies. We have transferred seven of these novel TE sequences to our partner companies. A member company is evaluating one TE's utility relative to their host biocatalyst to produce 8- and 10-carbon fatty acids. We are exploring the possibilities of establishing a start-up company around this TE technology, and this is being conducted in collaboration with our company members. Member companies have funded a project that integrates this TE technology with novel KASIII technologies that is being developed in T1.1, which in combination should be able to generate bi-functional chemicals.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems – Database Construction
[*This is an activity that supports all projects in Thrust 1, but it is being included here as an addendum to project T1.6.*]

Thrust: Research Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Peter Reilly	**02/23/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Peter J. Reilly
Graduate Students:
David C. Cantu (November 2008–present)
Yingfei Chen (February 2009–present)
Undergraduate Students:
Michael Forrester (January 2011–October 2012)
Hai Tran (January 2012–present)

Statement of Project Goals

We are constructing a database/webpage (ThYme, Thioester-active enzYmes, http://www.enzyme.cbirc.iastate.edu) that will contain all the amino acid sequences (primary structures) and three-dimensional structures (tertiary structures) of the enzymes in the eight enzyme groups involved in the fatty acid/polyketide synthesis cycle. Substrates of all of these enzymes contain thioester groups. Also included in ThYme will be the primary and tertiary structures of the acyl carrier proteins, which activate many of these substrates, and perhaps those of the thiolases and desaturases in the future. We have been using this information to write papers on the phylogeny and properties of these proteins. Furthermore, we are using quantum mechanics/molecular mechanics (QM/MM) to determine the mechanisms of a thioesterase and an enoyl reductase, both members of the fatty acid synthesis cycle.

Project's Role in Center's Strategic Plan

All center members will be able to access the primary and tertiary structures in ThYme. Since these data will be linked to many other databases, this should greatly simplify center members' tasks in gathering information on the enzymes on which they are working.

Fundamental Barriers and Methodologies

The largest barrier here is the sheer number of primary (300,000 at present) and tertiary structures (1,300 at present) that need to be gathered and ordered. In addition, new primary and tertiary structures are being discovered at an exponentially increasing rate (20% increase in numbers of the former and 10% increase in numbers of the latter annually), and they must be gathered by us as they are discovered and placed in other databases by other researchers. We have engaged the webpage development team in the ISU Information Technology Services to help in this effort. This has required extensive software coding that is essentially finished. This allows us to gather and to order data automatically, since there is far too much data to manage manually.

After gathering all primary and tertiary structures within an enzyme group with the Basic Local Alignment Search Tool (BLAST), we separate the sequences into families by their sequence differences using multiple sequence alignments with either Clustal or MUSCLE. With a few exceptions, members of different families are not related to each other, but instead are descended from different protein ancestors.

Achievements

We have completed classifying all eight enzyme groups [acyl-CoA synthases (ACSs), acyl-CoA carboxylases (ACCs), acyltransferases (ATs), ketoacyl synthases (KSs), ketoacyl reductases (KRs), hydroxyacyl dehydratases (HDs), enoyl reductases (ERs), and thioesterases (TEs)] plus acyl carrier proteins (ACPs), whose sequences and structures we have gathered. The ThYme database is fully operational and is accessible to center members and to the general scientific public.

Acyl-CoA synthases: There are five ACS families, with the first (ACS1) having approximately 40,000 sequences and being composed of enzymes with many different names and with Enzyme Commission (EC) designations widely spread around the EC 6.2.1 group, which encompasses acid-thiol ligases. They are produced by bacteria, eukaryotes, and archaea (ordered in decreasing numbers produced). ACS2 has a few hundred sequences, mainly 6-carboxyhexanoate-CoA ligases and pimeloyl synthases (EC 6.2.1.14), produced by bacteria and archaea. ACS3 has a couple of hundred bacterial citrate (pro-3S)-lyase ligases (EC 6.1.2.22). ACS4 has about 1,500 bacterial and archaeal phenylacetate-CoA ligases (EC 6.2.1.30). ACS5 has about 5,500 bacterial, eukaryotal, and archaeal sequences, mainly succinate-CoA ligases (ADP-forming) (EC 6.2.1.5). Only ACS1, ACS4, and ACS5 have members with known tertiary structures. The work on this enzyme group is done. There is not enough novel information here to justify a journal publication.

Acyl-CoA carboxylases: The ACCs are notable for having multi-domain structures, with the three main domains named for their roles [biotin carboxylases (BCs), biotin-carboxyl carrier proteins (BCCPs), and carboxyl transferases (CTs)]. BCs consist of one family of about 8,500 bacterial, eukaryotal, and archaeal sequences. BCCPs have about 5,500 bacterial, eukaryotal, and archaeal sequences, all in one family. There are two families of CTs. CT1 has approximately 10,500 bacterial, eukaryotal, and archaeal sequences, while CT2 has about 2,500 bacterial and eukaryotal sequences. All four families have known tertiary structures. Some ACCs have other domains. Furthermore, often other enzymes are found on the same protein chain, meaning that ACCs are not only multi-domain but are also part of multi-enzyme complexes.

Acyltransferases: ATs reversibly transfer CoA and acyl carrier protein (ACP) moieties linked by thioester bonds to acyl groups. There is only one AT family, consisting of approximately 10,500 primary structures, mainly in EC 2.3.1.39 (malonyl CoA-ACP transacylases). Nearly all ATs are produced by bacteria and eukaryota, with only a few archaeal producers listed. Many AT tertiary structures are known.

Ketoacyl synthases: There are five KS families, with KS1 through KS4 having some primary structures in common. KS1, KS3, and KS4 have very similar tertiary structures and therefore are in the same clan and appear to be descended from a common distant ancestor. KS1 has approximately 4,500 primary structures representing two enzymes, β-ketoacyl-[ACP] synthase I (EC 2.3.1.41) and β-ketoacyl-[ACP] synthase III (EC 2.3.1.180) produced by bacteria and a few archaea. KS2 has about 600 sequences and no known tertiary structure. Others have used homology modeling to predict its tertiary structure, which appears to be similar to those of KS1, KS3, and KS4. All KS2 members are produced by eukaryota, mainly plants; they have been given many names and several EC numbers. KS3 is a very large family, with about 20,000 sequences produced by bacteria, eukaryota, and a few archaea, and having many names and EC numbers. KS4 has about 3,500 eukaryotal and bacterial primary structures, many of them chalcone synthases or naringenin-chalcone synthases (EC 2.3.1.74). KS5 has almost 2,000 sequences, all produced by eukaryota, mainly animals, and being fatty acid elongases. This family has no known tertiary structure and seems to be quite different than the other four KS families.

Because of the importance of the KSs, we have split the five families into subfamilies. As mentioned previously, all members of a family have related primary structures, but members of one subfamily have sequences that are statistically different from those of another. KS1, KS2, KS4, and KS5 have 12, 10, 10, and 11 subfamilies, respectively. KS3 has 14 manually separated groups, the different treatment warranted by its size.

Ketoacyl reductases: There are four KR families. KR1 is the largest family among the eight enzyme groups in the fatty acid/polyketide synthesis cycle, having almost 60,000 sequences produced by bacteria, eukaryota, and archaea. Given the size of this family, its members have many names and EC numbers, but many are 3-ketoacyl-[ACP] reductases (EC 1.1.1.100). KR2 has about 4,000 bacterial, eukaryotal, and archaeal primary structures, many of them 3-hydroxyacyl-CoA dehydrogenases (EC 1.1.1.35), named after the reverse reaction. KR3 has a couple of hundred eukaryotal and bacterial 3-ketoacyl-[ACP] reductases that are part of fatty acid synthases. KS4 has about 4,500 bacterial and eukaryotal enzymes that are found with other enzymes in polyketide synthases. All four KR families have members with known tertiary structures.

Hydroxyacyl dehydratases: There are a great number of different dehydratases/hydratases, but only four of them act on substrates with thioester groups. Enoyl-CoA hydratases (EC 4.2.1.17) are found in HD1 and HD2, unrelated by sequence similarity. HD1 has about 2,500 members, nearly all produced by bacteria, with only a few produced by archaea and eukaryota. HD2 has over 9,500 primary structures, found in bacteria, eukaryota, and a few archaea. The almost 1,500 HD3 sequences, produced by eukaryota and bacteria, are mainly 3-hydroxypalmitoyl-[ACP] dehydratases (EC 4.2.1.61), found in multienzyme fatty acid synthases, plus peroxisomal multi-functional enzymes (EC 4.2.1.107). HD4 is comprised of a few hundred eukaryotal and bacterial 3-hydroxypalmitoyl-[ACP] dehydratases, also found in fatty acid synthases. HD5 consists of about 900 3-hydroxydecanoyl-[ACP] dehydratases (EC 4.2.1.60) produced almost solely by bacteria, while HD6 has almost 3,000 mainly bacterial undesignated hydroxyacyl-[ACP] dehydratases (EC

4.2.1.–]. All six families have at least one member with a known tertiary structure. In the past year, two further HD families, neither with known tertiary structures, have been added to ThYme. HD7 members, fewer than 500, are produced by bacteria and eukaryota and are not well defined. The almost 1,000 members of HD8 are produced solely by eukaryota and have been labeled as tyrosine phosphatase-like enzymes. Neither family has members with known tertiary structures.

Enoyl reductases: There are five ER families. ER1 has been absorbed into KR1, as the BLAST query sequence that produced this family appears to have actually been a KR. ER2 members are enoyl-[ACP] reductases (EC 1.3.1.9) produced by bacteria and some eukaryota. There are almost 2,500 of them. The couple of hundred enoyl-[ACP] reductase members of ER3 are produced by bacteria and eukaryota and are nearly all found in fatty acid synthases. The roughly 2,500 ER4 members are produced by bacteria and eukaryota and a few archaea and appear to be mainly parts of polyketide synthases. The few hundred eukaryotal and bacterial ER5 members are mainly *trans*-2-enoyl-CoA reductases (EC 1.3.1.38). Finally, ER6 is composed of almost 9,000 bacterial, eukaryotal, and archaeal 2,4-dienoyl-CoA reductases (EC 1.3.1.34). All families have members with known tertiary structures.

Thioesterases: There are 25 thioesterase families, in general not related by sequence similarity. Thirteen families are composed of acyl-CoA hydrolases, along with six families of acyl-ACP hydrolases, one family of protein-palmitoyl hydrolases, one family of protein-acyl hydrolases, two families of glutathione hydrolases, and two families that are yet unclassified. Only 12 of the 27 enzymes classified by function into EC 3.1.2.– are represented. Twelve of these families can be grouped into four clans by their slight similarity in primary structure but strong similarity in tertiary structure, with two clans having members with HotDog crystal structures, and with the other two clans whose members have α,β-hydrolase structures.

We have used multiple sequence alignments made with ClustalX or MUSCLE to further study TE14, because it contains enzymes that act on substrates containing short fatty acid chains. TE14 has ten subfamilies, four whose members are produced by plants and six whose members are produced by bacteria, with the members of each subfamily related to each other by their very similar sequences, but yet showing statistically significant differences from one subfamily to the next. We had hoped that thioesterase-catalyzed hydrolysis would liberate fatty acids and other molecular building blocks of the number of carbon atoms (4–8) desired by CBiRC, and that has occurred.

Our most recent work is to use quantum mechanics/molecular mechanics to predict the mechanism of a TE8 acyl-CoA thioesterase as it hydrolyzes the thioester bond of an acyl-CoA. In the human enzyme in this family, Asp65 acts as a base that activates a water molecule to attack the thioester carbon atom, hydrolyzing the substrate. HIP134 acts as an acid and donates a proton to Ser83, which in turn protonates the substrate thioester sulfur atom. In the transition state, the thioester and nucleophilic water molecule form a tetrahedral-like intermediate.

Acyl carrier proteins: All ACP primary and tertiary structures were gathered into the ThYme database. They are classified into 16 families by amino acid sequence similarity, with members of the different families having sequences with statistically highly significant differences. These classifications are supported by tertiary structure superposition analysis. Tertiary structures from a number of families are very similar, suggesting that these families may come from a single distant ancestor. Normal vibrational mode analysis was conducted on experi-

mentally determined freestanding structures, showing greater fluctuations at chain termini and loops than in most helices. Their modes overlap more so within families than between different families. The tertiary structure of an ACP family that lacked any known structures was predicted as well.

Other Relevant Work

There are other databases similar to ThYme, specifically ones for enzymes that are active on carbohydrates (CAZy) and peptidases (MEROPS). These gather thousands of hits from researchers around the world, and over the course of a year ThYme does also.

Plans for the Next Year

Since this is our last year in CBiRC, we have no further plans to disclose (except that ThYme will continue to be maintained).

Expected Milestones and Deliverables

Our expected milestones and deliverables are mainly listed above. The ThYme database will continue to grow indefinitely as new sequences and structures are automatically added to it. Its continued maintenance is a long-term commitment, as such resources cannot easily be abandoned without damage to the university's reputation and to researchers, here and elsewhere, who depend on it.

Member Company Benefits

CBiRC's industrial members will have full access to ThYme and will be able to use it to gain information on the various enzymes and proteins tabulated in it. It is impossible to estimate the number of person-hours and the production costs saved, or the market impact, but databases such as these save very large percentages, much greater than 50%, of the time needed for gathering information.

Commercialization / Technology Transfer

We have already worked out a protocol so that a CBiRC-affiliated company has downloaded all of ThYme. We are prepared to assist other companies, within and outside of CBiRC, to download all or parts of ThYme.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Bioinformatics [*This is an activity that supports all projects in Thrust 1, but it is being included here as an addendum to project T1.6.*]

Thrust: Research Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By: **Eve Wurtele**	Date *(in U.S. date format)*: **2/17/2013**	Reporting Period: **03/01/2012 to 02/28/2013**

ERC Team Members

Faculty:
Eve Wurtele, Department of Genetics, Cell and Development Biology, Iowa State University, (Project Leader), Basil Nikolau, Dept. of Biochemistry, Biophysics and Molecular Biology
Ling Li Department of Genetics, Cell and Development Biology, Iowa State University,
Postdoctoral Scholars: Wang Yi Department of Genetics, Cell and Development Biology, Iowa State University
Graduate Students:
Jon Hurst, Department of Genetics, Cell and Development Biology, Bioinformatics and Computational Biology Program, Iowa State University, Yves Sucaet, Department of Genetics, Cell and Development Biology, Bioinformatics and Computational Biology Program, Iowa State University, Alexis Campbell, Dept. of Biochemistry, Biophysics and Molecular Biology; Manhoi Hur, Human Computer Interactions.
Staff:
Nick Ransom, Program Analyst

Statement of Project Goals

Work with T1 researchers to create custom bioinformatics approaches for gene discovery. Integrate in-house 'omics data with existing database to provide a system-wide view of specialized genes. Create new approaches to help identify genes/proteins from plants and microbes important for the synthesis of carboxylic acids, bifunctional molecules, pyrones and for new functionality discovery.

Project's Role in Center's Strategic Plan

The bioinformatics tools developed in this project, and the genes identified, and models developed, provides a new approach for improving strains and achieving optimized product production of polyketides, and

Fundamental Barriers and Methodologies

This research project leverages the high volume 'omics data possible with new technologies.

Inherent barriers include factors such as interpretation of data due to compartmentalization of metabolites, accurate and large scale identification of metabolites, multigene families encoding proteins of promiscuous and overlapping function.

Achievement

- We created *Plant and Microbial Metabolomics Resource* (PMR, http://metnetdb.org/PMR/), a user-friendly metabolomics database with associated analysis capabilities that are critical to the accessibility and use of metabolomics data. As a CBiRC private resource (and a public resource), this capability facilitates discovery and development of testable hypotheses. We have also created a prototype for transcriptomics/metabolomics co-analysis within the context of PMR.
- We have expanded our direct pipeline (2011) to access public transcriptomics data by automating processes for generating huge (30,000 x 30,000 gene) matrices. As a test case, we have successfully applied this pipeline to generate a matrix from >7,000 Arabidosis transcriptomics samples in the public database Array Express.

Plans for the Next Year

- In year 6, we will design targeted transcriptomics studies to identify candidate genes for synthesis of desired functionalities. We will leverage experimental data generated by T1 CBiRC researchers to integrate new information that can be used for hypothesis development. We intend to begin the implementation of a network information-genetic algorithm approach. The algorithm will provide novel methods to develop experimentally testable hypotheses based on metabolomics, transcriptomics data in the context of a metabolic model. We plan to conduct a short workshop in bioinformatics analysis using PMR and other tools for interested students and other researchers.

Expected Milestones and Deliverables

- Integrate information based on targeted transcriptomics studies to expand and enhance identification of genes that encode proteins of functionalities that can be applied to synthesis of desired polyketides.
- Increase computing speed of transcriptomics-metabolomics comparisons to enable real-time analysis of massive datasets locally, using an approach that incorporates NoSQL- and GPU.
- Draft prototype for combined network information-genetic algorithm analysis.

Member Company Benefits

The metabolomics database and associated statistical tools would provide an excellent tool for industry researchers in their analysis of factors that contribute to composition in relation to polyketide (acetate-based) test beds. The bioinformatics software and metabolomics database can be used by industry and applied to analysis of targeted manipulation of a wide range of compounds.

Commercialization / Technology Transfer

A provisional patent (no. 61/446.469) has been granted (based on an associated NSF-funded project) for a gene that controls accumulation of lipids, starches and oils.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: 02-13S2 - Structural and Substrate-binding Studies of KASIII Enzymes to Understand Their Evolutionary Relationship with PKS Enzymes

Thrust: Research Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*: **02/28/2013**	Reporting Period: **03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Basil Nikolau, Iowa State University

Other Faculty: Joseph P. Noel, Salk Institute for Biological Studies

Graduate Students: Shivani Garg, Iowa State University

Postdocs: Charles Stewart, Salk Institute for Biological Studies

Statement of Project Goals

This collaboration between Shivani Garg (Nikolau group, ISU) and Charles Stewart (Noel group, Salk Institute) aims to examine the substrate binding of KASIII and PKS enzymes using structural and thermostability data to eventually enable understanding of their evolutionary divergence and the molecular basis of iterative versus non-iterative use of substrates by PKS and KASIII respectively.

Project's Role in Center's Strategic Plan

Knowledge gained from these studies will benefit 1) Stewart's efforts to understand the relationship between KASIII's and PKSs; 2) Garg's efforts to understand the structural basis for diverse functionalities of novel KASIII enzymes; and 3) CBiRC's aim of developing novel biocatalysts.

Foreign Collaborations

Not applicable.

Achievements

This project was only recently funded through the Center's Student-led Research Grant program. Consequently, there are no achievements to report at this time. The work is not scheduled to commence until 3/1/2013, after the annual report has been submitted.

Expected Milestones and Deliverables

Milestones:

1. Crystallization screen of several catalytically diverse KASIII enzymes for microbial production of unusual fatty acids.
2. Isothermal substrate-binding data of KASIII enzymes with different substrates.

Deliverables:

1. Understanding of evolutionary relationship between KASIII and PKS enzymes.
2. Understanding of "design rules" governing functionalities of novel KASIII enzymes.

Technology Impacts

Potential impacts of this project will include:

1. Two manuscripts detailing the studies leading to the above-mentioned deliverables.
2. Structural and substrate binding results of this project will lay a foundation for understanding the molecular basis of non-iterative versus iterative chemistry in KASIII and type III PKSs and exploiting this knowledge to develop novel molecules.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **31-12F1** - Combinatorial integration of novel biocatalysts that intercept fatty acid synthase to form innovative bi-functional carboxylic acids

Thrust: Research Thrust 1 - New Biocatalysts for Pathway Engineering

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Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Marna Yandeau-Nelson	**02/16/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Marna D. Yandeau-Nelson, Iowa State University
Other Faculty: Basil J. Nikolau, Iowa State University
Graduate Students: Shivani Garg and Fuyuan Jing, Iowa State University
Undergraduates: Colin Hueser, Iowa State University
Other Personnel: Huanan Jin (Post-doc), Iowa State University

Statement of Project Goals

This project will develop the biocatalytic technology for the fermentative production of bifunctional carboxylic acids of specific chain lengths. This technology will be based on the previous and ongoing characterization of diverse KASIIIs (Thrust 1 Project 1: 3-ketoacyl-ACP Synthase) that catalyzes the formation of the first new carbon-carbon bond in the process of fatty acid biosynthesis, and diverse TEs (Thrust 1 Project 6: Thioesterases) that catalyze the termination of fatty acid biosynthesis. We will exert control over the fatty acid structures and produce omega-functionalized carboxylic acids by utilizing variant KASIIIs that can use different starter and extender substrates in fatty acid biosynthesis. This biocatalyst will be combined with TE variants that show different substrate specificities, and terminate the process of fatty acid biosynthesis at shorter chain lengths than normal. Thus, by combining these two biocatalytic systems, we will have a combinatorial means of chemically functionalizing the products of fatty acid biosynthesis, and producing functionalized carboxylic acids of different, but specific chain lengths. The primary goal of this project is to demonstrate enhanced production of these bi-functional, chain length-specific carboxylic acids in a scalable system.

Project's Role in Center's Strategic Plan

By combining the KASIII biocatalyst with a TE with known substrate specificity, we can produce omega-functionalized fatty acids of specific chain lengths. This proposed work leverages the accomplishments achieved in Projects T1.1 and T1.6 within CBiRC Thrust 1, which focus on the characterizations of novel KASIII and TE enzymes, respectively. The work proposed herein will allow us to advance the goals of CBiRC by integrating the technology platforms developed by CBiRC for KASIII and TE to produce carboxylic acids with multiple desirable characteristics, combining specific bi-functionalities and specific chain lengths. This

will open new opportunities to CBiRC for innovation in bi-functionality, including the production of homogeneous sources of fatty acids (i.e. a specific chain length) with diverse functionalities (e.g. aromatic, halogen, nitrogen or sulfur groups on the terminal end). The ability to produce fatty acids with diverse bi-functionality will broaden the array of monomer feedstocks for the production of "green" chemicals via chemical catalysis by researchers within Thrust 3.

Fundamental Barriers and Methodologies

The broad substrate specificities conferred by the majority of TE and KASIII that have previously been characterized are a fundamental challenge to producing homogenous sources of bifunctional carboxylic acids. To overcome this barrier, we are systematically characterizing TE and KASIII biocatalysts that are derived from diverse biological systems and are identifying those enzymes with near mono-substrate specificity. Moreover, we are integrating into this project TE variants that have been engineered in Project T1.6 to produce near-homogenous pools of carboxylic acids of specific chain lengths.

Achievements

- Selected three TEs with near mono-specificities to short or medium chain acyl-ACPs.
- One vector has been successfully engineered and sequence confirmed. Two vectors are in the final stages of construction. Transformation into the engineered bacterial system #1 is in progress.
- All primers have been designed and PCR executed for cloning one of the three TEs into bacterial system #2. Vector construction is in progress.

Other Relevant Work

Within Basil Nikolau's lab, an additional 35 novel acyl-ACP thioesterases are being characterized in *E. coli* (Project T1.6) and *in vitro* functional characterization of a set of ~30 diverse KASIII enzymes (Project T1.1) is in progress. These new biocatalysts will increase the numbers of testable KASIII-TE combinations within our engineered bacterial systems to achieve combination(s) of TE and KASIII enzymes that produce the most homogenous (i.e. chain length-specific) source of bi-functional carboxylic acid.

Plans for the Next Year

- Build individual constructs for each of five TEs, which have particular substrate specificities. Constructs will be built and transformed into two separate bacterial expression systems engineered to produce bi-functional carboxylic acids.
- Evaluate newly characterized TEs (CBiRC Project T1.6) and KASIIIs (CBiRC Project T1.1) and channel those with near mono-specificity for a chain-length or omega-functionality, respectively, into the vector construction pipeline.
- Test several TE/KASIII combinations in two engineered bacterial systems for the production of bi-functional carboxylic acids.

Expected Milestones and Deliverables

- Engineered bacterial host strains that harbor one of three KASIIIs that act on branched starter substrates and one of 5-7 TE enzymes with diverse specificities for chain length (4-, 6-, 8-carbons, etc.).
- Engineered bacterial host strains that harbor one of three KASIIIs that act on branched

starter substrates and one of 3-5 TE enzymes with diverse specificities for functionality within the acyl chain.
- GC-MS profiles of carboxylic acids produced by strains expressing specific combinations of unique KASIII and TE enzymes.
- Combination(s) of unique TE and KASIII enzymes that produce bi-functional carboxylic acids in the bacterial host.

Member Company Benefits

Successful completion of the project will provide CBiRC and CBiRC's IAB members with an important proof of principle for the production of homogeneous sources of bio-based bi functional feedstocks by employing multiple biocatalysts from the fatty acid biosynthetic pathway. This project was in part, built upon the market studies and industry input garnered as a part of the NSF I-Corps grant awarded to Shivani Garg, Basil Nikolau and Peter Keeling to explore the commercial potential of KASIII-based technology to make bifunctional fatty acids. Through the I-Corps program, we have learned that branched chain acids, which perform better at lower temperatures, will be very attractive to numerous surfactant and lubricant companies, whereas the polymer industry would be attracted to hydroxylated or amine-carboxylic acids, because they are ideal monomers for bio-based polyesters and polyamides. Therefore, the innovations developed through this project will likely facilitate partnerships with CBiRC member companies who function in the surfactant, lubricant and polymer arenas.

Commercialization / Curriculum Impacts

The initial demonstration of production of homogeneous pools of bi-functional carboxylic acids in this project will allow us to identify opportunities that are best positioned for development towards commercialization of such bio-based products, with primary focus, at least initially, on the surfactant, lubricant and polymer industries.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.1A -** Strain Construction and Optimization in *E. coli*

Thrust: Research Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Ka-Yiu San	**02/19/2012**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Ka-Yiu San, Rice University
Other Faculty: Ramon Gonzalez, Rice University; Laura R Jarboe, ISU
Research Scientists: Xiujun Zhang, Wei Li, Rice University
Graduate Student: HongI Han, Rice University; Ping Liu, Liam Royce, ISU

Statement of Project Goals

The goal of the project is to develop metabolic engineering tools to design and construct efficient *Escherichia coli* strains for high level production of biochemical intermediates from glucose.

Project's Role in Center's Strategic Plan

The project plays a central role in bridging the other two research thrusts. Specifically, the project focuses on constructing efficient microbial systems to produce biochemical intermediates, which will be used in Thrust 3, using knowledge and materials from Thrust 1. Specifically, genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2.

Fundamental Barriers and Methodologies

Successful development of efficient strains for high level production of biochemical intermediates from glucose requires several issues to be addressed. The first challenge is to introduce new functional pathways into *E. coli* to produce the targeted product. Since most of the genes involved in these pathways are from plants, the expression of biologically active enzymes in *E. coli* may require additional effort. The enzymes might have to be modified in order to function efficiently in *E. coli*. Furthermore, the production strain must be designed to be able to channel cellular resources, such as carbon precursors, cofactors and energy, for the synthesis of the desired product. In this project, molecular biology and metabolic engineering techniques (including co-factor engineering) will be developed and used to overcome these challenges. More importantly, strain development is an iterative process, knowledge learned from other projects, such strain characterization and omics studies, will be used to provide insight in designing additional strains with improved performance.

Achievements

1) Carboxylic acid

1A) Construction of mutation strains with Δgpm and others

Mutant strains were constructed in collaboration with other T2 thrust members. The mutations were identified as potential fatty acid production bottleneck based on metabolic flux and in silico analyses. One

potential mutant strain to be examined is the glycolytic phosphoglycerate mutases (PGM) which was predicted that down-regulation of this pathway will lead to an increase in the fatty acid production. It has been shown that there are two non-homologous isofunctional enzymes showing PGM activity in *E. coli.* Deleting the cofactor dependent PGM (*ΔdPGM* or *ΔpgmA*) has been shown to have detrimental effect on forming colony on plate; the *ΔdPGM* mutant strain failed to form colonies after 24 h growth and colonies appeared only between 48 and 72 hrs (Jeremy et al., PLoS ONE 5:e13576). We therefore decided to focus on constructing a cofactor independent PGM (*ΔiPGM* or *ΔpgmM*) mutant strain using Warner method with ML103 (MG1655 *ΔfadD*) as the base strain. The resulting mutant strain was confirmed with genomic PCR and sequencing.

1B) Construction of strains carrying both short and long chain acyl-ACP thioesterases to improve shorter medium chain fatty acid production

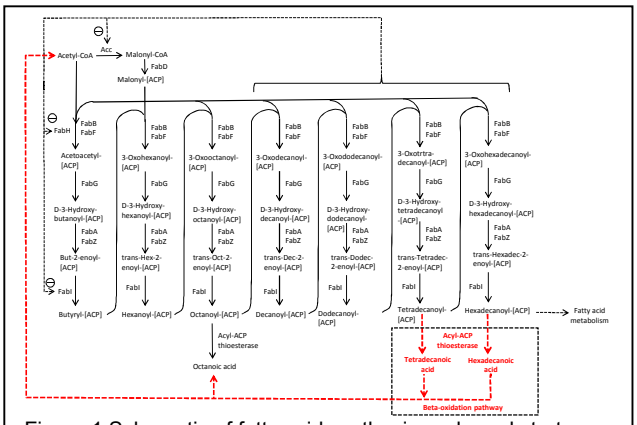

Figure 1 Schematic of fatty acid synthesis cycle and strategy to alleviate acyl-ACP feedback inhibition effect

Design Rationale: Since we have been able to produce longer medium chain fatty acids (C14 and C16) at high yields and high rates, our results suggest that *E. coli* has the metabolic capacity to produce fatty acids at high efficiency that are close to the end of its synthesis cycle. However, the situation will be different for shorter medium chain fatty acid production, such as octanoic acid. First, the acyl-ACP thioesterase has to pull the shorter medium chain fatty acid in the middle of the synthesis cycle. Second, literature reports showed that the accumulation of longer chain acyl-ACP has negative feedback effect on several fatty acid synthesis pathways (see Figure 1). A schematic depicting the concept of alleviating potential acyl-ACP feedback inhibition effect is shown in Figure 1. The co-expression of short and long chain acyl-ACP thioesterases has three effects: 1) the shorter acyl-ACP thioesterase will lead to the synthesis of free short chain fatty acid; 2) the longer acyl-ACP thioesterase will lead to lower longer long chain acyl-ACP concentrations; 3) the longer acyl-ACP thioesterase will provide a pulling effect of the fatty acid precursor acetyl-CoA into the fatty acid synthesis cycle. To further increase the carbon yield, the longer chain length free fatty acid can be recycled through the fatty acid beta-oxidation pathway.

Strain construction: A strain was constructed to test this idea using three plasmid. One plasmid, pXZcp88, carries an acyl-ACP thioesterase specific to shorter chain length acyl-ACP. The expression of this thioesterase is under the control of an IPTG inducible *trc* promoter system. The second plasmid, pDHC29, is an expression vector which is compatible to the first plasmid and will serve as a control. The third plasmid, pDHC29-18 carries an acyl-ACP thioesterase specific to longer chain length acyl-ACP using pDHC29 as the cloning vector. The expression of this thioesterase is under the control of an IPTG inducible lac promoter system.

Other Relevant Work: Since the biosynthesis of fatty acid requires significant quantity of the cofactor NADPH and acetyl-coA (for ex., each fatty acid elongation cycle requires two molecules of NADPH), results and knowledge from another project aiming to design and construct efficient strains with increased NADPH availability for chiral compound production may be useful in the current project to increase fatty acid levels.

Plans for the Next Years: Further fine-tuning of the host strains and expression vectors will be carried out in the coming years. Furthermore, the design, construction and characterization of more advanced modified acyl-ACP thioesterases leading to higher specificity and productivity will be pursued. The design of the second generation production strains will be based on the characterization, omics and in silico studies. In

addition, single plasmid carrying multiple genes will also be constructed to study the effect of introducing multiple genes into the system (integration into the chromosome if necessary). These multiple genes constructs will be performed after initial proof-of-concept experiments with multiple compatible plasmids each carrying a single gene. Finally, efforts will also be put on targeting the production of shorter chain length carboxylic acids and improve the purity of the carboxylic acids (i.e., with one predominant chain length rather than a mixture). As such further development of new and more efficient acyl-ACP thioesterases (in terms of activity and specificity) as well as strains is necessary. Finally, we will continue to construct strains for bifunctional fatty acid production.

Specifically, we plan to: 1) built on current framework to integrate various genetic and metabolic manipulations to further increase the product titer; 2) perform further metabolic engineering studies to improve current strain to increase product/glucose yield; 3) develop new strains based current framework to produce shorter chain length carboxylic acids efficiently; 4) design, construct and characterize more efficient thioesterases for efficient production of shorter chain length carboxylic acids; 5) perform characterization studies to gain insight into the mechanisms leading to efficient strain and thioesterase constructs; 6) incorporate mutations that confer fatty acid tolerance into the fatty-acid producing strain (dependent on identification of those mutations, described in bioinformatics report; 7) address metabolic bottlenecks identified in the simultaneous flux/transcriptome/proteome study.

Expected Milestones and Deliverables: The deliverables will be strains and vectors for the expression of biologically active enzymes for short/medium chain fatty acid biosynthesis. We will improve *E. coli* fatty acid productivity through strain design and construction aiming to attain higher titer, yield and production rate. In addition, we will design, construct and characterize modified acyl-ACP thioesterases for the efficient production of purer free fatty acid at higher rates.

Member Company Benefits: The knowledge and constructs (plasmids, strains and acyl-ACP thioesterases) being developed in this project will be useful to member companies.

Commercialization / Technology Transfer

We are actively engaging with Center members and other companies, including start-ups, for potential licensing and commercialization of the carboxylic acid production technologies (1 US patent application, 1 PCT application and 2 provisional patent applications).

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T2.1B - Strain Construction and Optimization in *S. cerevisiae*

Thrust: Research Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Nancy A. Da Silva	**02/14/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Faculty: Nancy Da Silva (UCI), Suzanne Sandmeyer (UCI)
Instructor: Brian Sato (UCI)
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Undergraduate Students: Marc Kryger (UCI)

Statement of Project Goals

The goals of the work are to design and construct *Saccharomyces cerevisiae* strains (and other yeast species) for high-level production of carboxylic acids and pyrones from glucose, and to develop the necessary genetic tools to efficiently engineer the strains.

Project's Role in Center's Strategic Plan

The goal is to construct microbial strains to produce test bed chemicals, including carboxylic acids and pyrones. These test beds will provide opportunities to integrate all three research thrusts. Genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the products from Thrust 2 will serve as precursors for the synthesis of α-olefins, dienes, and other compounds by Thrust 3, the Chemical Catalysis group.

Fundamental Barriers and Methodologies

The synthesis of short chain fatty acids requires access of novel thioesterases (TEs) to the growing fatty acid chain. This is precluded in *S. cerevisiae* by the complex and closed structure of the native fatty acid synthase (FAS). To address this, we are introducing heterologous Type I and Type II FAS systems that allow enzyme access. The introduction of a second pathway also allows us to balance the synthesis of required fatty acids for cell viability and of the desired short-chain products. Synthesis of high levels of carboxylic acids and pyrones requires high-level production of active synthase enzymes, and sufficient precursor synthesis. Strains are being engineered and evolved for this purpose. In addition, the project requires the ability to efficiently construct and modify strains by introducing multiple genes. To address this, we have developed a robust set of expression vectors for metabolic pathway engineering in *S. cerevisiae*. We have also extended our efforts to the oleagionous yeast *Yarrowia lipolytica*, a promising yeast for high-level polyketide synthesis.

Achievements

During the first four years of the Center, our efforts focused on the development of a *S. cerevisiae* toolkit for strain construction, and on the manipulation of fatty acid, methyl ketone, and pyrone synthesis in yeast. We also initiated work with the oleagionous yeast *Yarrowia lipolytica*. During the fifth year, we have (1) developed genetic backgrounds and expression plasmids to facilitate work in *Yarrowia lipolytica*, (2) expanded the toolkit for *S. cerevisiae* metabolic engineering, (3) constructed strains for the expression of exogenous FAS systems in *S. cerevisiae* and introduced promising thioesterases for short chain fatty acid synthesis, (4) engineered strains for high level synthesis of pyrones, and (5) engineered *S. cerevisiae* for increased synthesis of required precursors.

(1) Development of genetic backgrounds and expression plasmids to facilitate work in the oleaginous yeast *Yarrowia lipolytica (Yl)*

The goal of this work is to take advantage of a yeast that naturally makes high levels of lipids to generate compounds of industrial interest. For this purpose we have chosen the oleaginous yeast *Yarrowia lipolytica* (*Yl*) that naturally stores lipids rather than polysaccharides. In addition, it is reported to use more diverse carbon sources than *Saccharomyces cerevisiae* (*Sc*). *Yl* is sufficiently diverged from *Sc* that it is necessary to develop independent expression plasmids and marker genes to undertake molecular genetic manipulation. In addition, it has a high level of nonhomologous recombination complicating gene targeting strategies. We constructed a shuttle vector (pJY3731) containing the pUC18 backbone, bacterial beta lactamase gene for ampicillin resistance, the *Yl URA3* selectable marker, *Yl* CEN/ars18 origin of replication, and expression site containing the *Yl TEF1* promoter driving hrGFP followed by the *Sc CYC1* terminator. Previous *Yl* RNA-seq data were clustered to identify twelve promoters representing ones with strong constitutive expression or increased expression at a late stage of growth. The *Yl TEF1* promoter was replaced with the promoter set to create a series of plasmids predicted to have different levels of expression of hrGFP and these were evaluated for expression using flow cytometry. Overall there was a range of absolute activity, but on average the cluster analysis prediction was confirmed by promoter performance.

In order to express heterologous genes from our shuttle plasmid it was necessary to construct mutant strains appropriate for genetic marker selection. Because of the high rate of off-target integration, we tested to define the relative frequencies of homologous and non-homologous recombination. This was reflected as the ratio of *URA3* knock-out (homologous recombination) to *URA3* knock-in (non-homologous plus homologous recombination). These experiments showed homologous recombination occurred at a frequency of ~8% with constructs having 700 bp flanking homology. Homologous recombination increased with length of flanking homology (200<500<700=1000 bp). Deletion of components of non-homologous recombination is underway to potentiate homologous recombination using PCR fragments. To expand the set of usable markers, the *URA3* gene flanked by 1 kb upstream and downstream of *Yl LEU2* was used to knock out that gene.

We are currently using these vectors to express heterologous pyrone synthase, and these strategies to construct strains with elevated lipid production.

(2) Expansion of toolkit for metabolic engineering in *Saccharomyces cerevisiae*

We previously completed construction of a toolkit of yeast pXP vectors to allow the

combinatorial expression of metabolic genes in *S. cerevisiae*. A paper describing the initial vectors was published in *Yeast* in October 2011. We have completed the expansion of this vector set to include three inducible/repressible promoters: P_{GAL1}, P_{ADH2}, and P_{CUP1}. They are available as high- and low-copy plasmids, and are designed with reusable selectable markers that enable PCR amplification of cassettes, sequential chromosomal integration, and subsequent simultaneous excision of the markers. A second paper describing these vectors was published in *Yeast* in November 2012. A new PCR-based integration strategy has also been designed to integrate tandem genes (with different promoters and terminators) into a single locus in order to optimize time and resources. The full set of vectors and expression loci characterized facilitate rapid and systematic combinatorial expression of pathway genes for metabolic engineering, and have been used extensively in CBiRC projects 1B and 2B.

(3) Development of *S. cerevisiae* strains for synthesis of short chain fatty acids

To avoid the inherent limitations of the yeast FAS and to optimize short chain fatty acid production, we have investigated the expression of heterologous FAS systems (mammalian and *E. coli*) in *S. cerevisiae*. The non-native FAS systems allow access by the thioesterases required for short chain synthesis, enable easier optimization of the host by avoiding native regulatory control, and allow utilization of separate FAS systems for host cell requirements and product synthesis. Previously we showed that expression of active holo-mFAS was sufficient to complement a yeast *FAS2* knockout, allowing growth in the absence of exogenous fatty acid supplements. We integrated the auxiliary genes and engineered the yeast expression system for enhanced stability and robustness. This functional replacement is very promising as the mFAS can be used with thioesterases that allow short chain synthesis. For the synthesis of short chain fatty acids, we have combined novel thioesterases (TEs) from Thrust I and two TEs from the literature with our new heterologous yeast systems. To test these TEs for short chain fatty acid synthesis, the TE domain was removed from the mFAS, and the new TEs are carried on high-copy plasmid vectors for testing. In addition, we have created two mutant mFAS constructs containing a short chain thioesterase domain by fusing the TE directly to the mFAS via a linker. These short chain TE domains replace the native TE domain and allow the direct shuttling of acyl-CoA substrates from the adjacent ACP domain. Total *in vivo* short chain fatty acid production increased 19-fold over controls. A 32-fold and 21-fold increase was observed for C_8 and C_{10} levels over controls (Project 2B).

To increase the *in vivo* production of short chain fatty acids we have developed and characterized strains for the down-regulation of the native yeast FAS, and that prevent the oxidation of fatty acids, secrete the fatty acids into the growth medium, and have reduced proteolysis activity. In addition, three separate phosphopantetheine transferases, capable of forming holo-mFAS, have been identified and tested for *in vivo* short chain fatty acid production. We retain the native yeast FAS in our strains to supply the necessary C_{16} and C_{18} length fatty acids for cellular function and growth. However, the yeast FAS competes with our heterologous mFAS for the same required substrates and cofactors. A strong constitutive promoter was integrated in front of the yeast *FAS2* gene, removing native transcription control, and fatty acids levels were monitored by use of a GC-MS. Additional promoters are currently being investigated for their effectiveness in controlling the timing and level of the native yeast FAS expression. *S. cerevisiae* also constantly undergoes lipid turnover through β-oxidation. We have created a strain incapable of lipid turnover by removing an acyl-synthase, a fatty acid activator and a fatty acid transporter. This strain is currently being combined with

our heterologous mFAS system and short chain fatty acid levels will be monitored with GC-MS. Fatty acids can passively diffuse into and out of the extracellular space or be actively secreted/imported through vectorial acylation. We have created seven different strains deficient in the vectorial acylation process and removed two fatty acid activators. These strains are currently being tested for intracellular and extracellular fatty acid levels at exponential and stationary phase. Additionally, we have successfully reduced proteolysis activity by transferring our heterologous FAS systems into a protease deficient host, BJ5464.

In parallel, we have focused on introducing the *Escherichia coli* fatty acid pathway as the separate proteins allow the greatest flexibility for manipulation. Previously we expressed all nine required genes in active form in yeast and confirmed activity with *in vitro* experiments. We have now integrated, using new integration strategies, eight of the nine mandatory type II FAS genes into a single strain, with expression of the thioesterase on a plasmid. Thioesterases from different hosts have been introduced into this strain. To test for the activity *in vivo* of the complete type II FAS system expressed in *S. cerevisiae,* two different assays have been used. First, the total fatty acid production and fatty acid profile of each of the strains constructed have been measured using GC-MS and GC-GC-MS. Second, growth complementation studies have been carried out. The native yeast FAS2 gene was knocked out to test if the expression of the active type II FAS was sufficient to complement the lack of native fatty acid synthesis. Complementation occurred, allowing the cells to grow in the absence of exogenous fatty acids (Project 2B). We are currently characterizing our strains and engineering them further to increase the production of short-chain fatty acids.

(4) Engineering of *S. cerevisiae* strains for high-level synthesis of pyrones

The enzyme 2-pyrone synthase (2-PS) from *Gerbera hybrida* (Thrust 1) has been cloned into pXP expression vectors under the control of a variety of strong promoters. These plasmids have been transformed into our yeast host strains for analysis of pyrone synthesis levels. Similar expression systems have been constructed using forty-one 2-PS mutant enzymes developed in collaboration with the Noel laboratory (Thrust 1), expanding off the six in the past reporting year. Results for twenty-three of these mutants have been provided (Project 2B), and the next eighteen are currently being evaluated.

Protease deficient strains were constructed by knocking out two common proteases, *PEP4* and *PRB1*. Following construction of *pep4Δ*, *prb1Δ*, and *pep4Δ prb1Δ* strains, pyrone synthesis was evaluated using the plasmid constructs described. A computational approach using the OptKnock procedure included in the COBRA Toolbox (http://opencobra.sourceforge.net) was used to suggest metabolic engineering strategies to improve the strain's ability to produce pyrones, specifically precursor availability. The use of this toolbox provided pathways to be manipulated, which included both pyruvate metabolism and fatty acid synthesis in aerobic, glucose-fed cells. The algorithm has been extended to identify additional interventions for acetyl-CoA target increases. These interventions significantly impact carbon flux towards important precursors by eliminating gluconeogenesis, energy storage, pentose biosynthesis, glycerol production, and cofactor transport. The construction of 11 single gene knockout strains, or the inhibition of fatty acid synthesis through the use of cerulenin, increased pyrone productivity (mg/L/OD) by as much as ~138% (Project 2B). Strains carrying a combination of these single gene deletions have been constructed to provide additional improvements in pyrone synthesis.

By combining the protease knockouts, optimum promoter, optimum expression system, and best 2-PS mutant, we have increased our pyrone titer to 1.74 g/L, a theoretical yield of 37%. Current efforts focus on developing strains to further increase titer and yield, and on developing an optimum expression system for use in minimal culture medium.

(5) Strain engineering for increased precursor synthesis

To engineer strains for increased production of fatty acids and related compounds, we have knocked out specific regulatory and pathway genes, upregulated genes for the synthesis of important precursors, and evolved strains for high-level fatty acid synthesis and for increased resistance to short chain fatty acids. The key pathways are shown in Figure 1.

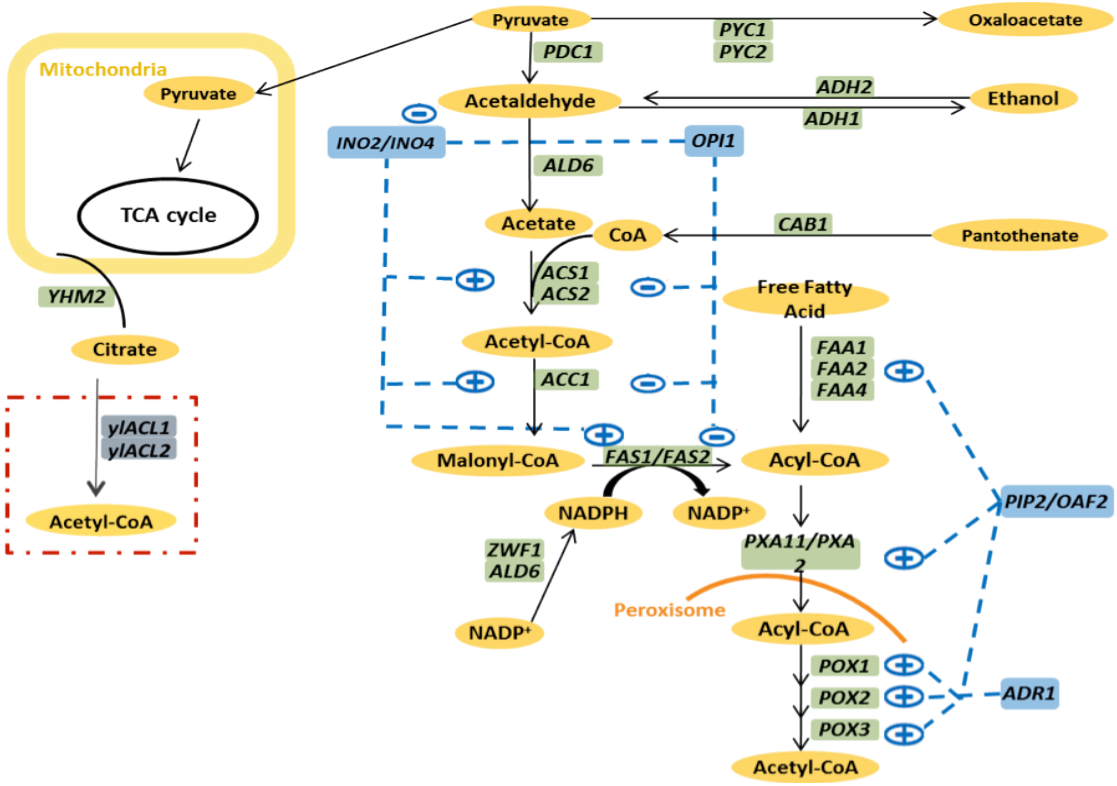

Figure 1: Engineering of Critical Pathways

The focus over the past year has been engineering strains carrying a combination of genes leading to increased production of precursors (e.g., CoA, acetyl-CoA, malonyl-CoA). Two promoters have been incorporated and the strains are being evaluated for the increased synthesis of a model polyketide (6-MSA), fatty acids, and pyrones. As an alternative approach to increase the pool of acetyl-CoA, we are utilizing non-native enzymes.

Other Relevant Work

Relevant similar work is also being conducted within CBiRC using *E. coli* as the model microbial system. In combination, the research will evaluate two promising microbial systems for the synthesis of the precursor compounds required for the Center's goals. To our knowledge, similar work on the use of heterologous FAS systems in yeast for the synthesis of short chain compounds is not taking place outside of this Center. Similarly, comparable work on pyrones is not underway.

Plans for the Next Year

Increase titer and yield of short chain fatty acids and pyrones:

Optimize yeast system for synthesis of short chain fatty acids. This work will involve selection of the optimum heterologous FAS system, removal of pathway bottlenecks, and modification of strains based on information from the Omics, Flux, and Bioinformatics groups.

Optimize yeast system for synthesis of pyrones. This work will involve selection of the optimum enzyme for use in the yeast host, removal of pathway bottlenecks, and modification of strains based on information from the Omics, Flux, and Bioinformatics groups.

Develop Y. lypolytica expression strains. The work will include the development of tools and strains for both short chain fatty acid and pyrone production. Transfer of pathways to *S. cerevisiae* may prove advantageous in this non-oleaginous yeast species.

Expected Milestones and Deliverables

Effective tools for metabolic engineering in yeast

Strains engineered with heterologous fatty acid synthase systems that allow manipulation of the synthesis pathway

Strains engineered for the high-level synthesis of limiting precursors

Strains engineered for high-level pyrone synthesis

Member Company Benefits

The benefits for the Center's industry members are the development of vectors and strains for the high-level synthesis of carboxylic acids and pyrones. In addition, strains with increased levels of the CoA precursors will be useful for a variety of products. Efficient metabolic engineering tools and methods developed will also be beneficial.

Commercialization / Technology Transfer

We have discussed collaborations with both Lesaffre and NCAUR for the pyrone research. Genes and vectors are being transferred to NCAUR (under a Materials Transfer Agreement) for studies in industrial yeasts. Further opportunities for technology transfer and commercialization are expected from the work.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T2.2A - Strain Characterization and Optimization in *E. coli*

Thrust: Research Thrust 2 - Microbial Metabolic Engineering

Pursuant to guidelines for preparing ERC annual reports, three- to five-page summaries for all projects with direct support, organized by thrust and/or education/outreach program area, must be provided. Project summaries do not have to be included for proprietary projects where such a summary would compromise the sponsor's interests. A project summary should also be provided for each supplementary and special-purpose award received by the ERC. In general, project summaries are NOT required for *associated* projects; rather, only an abstract is required in these cases. However, for Gen-3 ERCs, foreign partner associated projects may include a project summary rather than only an abstract if the project is of particular importance to achieving the vision of the center. Each project summary should contain all the sections shown below.

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Ka-Yiu San	**02/14/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Ka-Yiu San, Rice University
Other Faculty: Ramon Gonzalez, Rice University; Laura R Jarboe, ISU
Research Scientists: Xiujun Zhang, Wei Li, Rice University
Graduate Student: HongI Han, Rice University; Ping Liu, Liam Royce, ISU

Statement of Project Goals

The goal of the project is to characterize the production strains under various operating conditions and to further optimize their performance. The results/data from this project will be used to design omics experiments and to guide further genetic manipulations for strain improvement.

Project's Role in Center's Strategic Plan

The characterization study will assess the effect of genetic manipulations on the performance of the production strains and will provide important data/inputs for improving strains and achieving optimized product production. Specifically, genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2.

Fundamental Barriers and Methodologies

1) Carboxylic acids

1A) Effect of host strain and introduced pathways: Host strain and the introduced pathway may have significant effect of the rate and extent of fatty accumulation. In addition, the genetic background of the host strains may also affect the fatty acid chain length distribution. Extensive experimentation is needed to provide insight into the interaction between productivity and production strains.

1B) Effect of acyl-ACP thioesterase and its derivatives: The ability to express functionally active acyl-ACP thioesterase in *E. coli* has shown to have dramatic effect on the accumulation of free fatty acids. Effort will be need to gain a better understanding and develop the ability to design and construct efficient acyl-ACP thioesterase in *E. coli*.

1C) Effect of operating conditions: Operating conditions such as temperature, dissolved oxygen concentration, pH, as well as medium composition often play an important role in process performance.

Extensive experimentation will be carried out to quantify these effects.

Achievements

1) Carboxylic acid

1A) Fatty acid production by the Δgpm mutant strains

The parent strain ML103 and the ML103 *ΔiPGM* mutant strain carrying the plasmid pXZ18 and plasmid pXZ18Z were compared.

Both strains, ML103(pXZ18) and ML103(pXZ18Z), produce more free fatty acids than theirs *ΔiPGM* counter parts ($p<0.05$) at 48 hours (Figure 1A). The parent strain ML103(pXZ18) produces about 9% more fatty acids than that of the ML103 *ΔiPGM* (pXZ18) mutant strain. Similarly, The parent strain 103(pXZ18Z) produces about 19% more fatty acids than that of the ML103 *ΔiPGM* (pXZ18Z) mutant strain. These results suggest that deletion of the *iPGM* gene leads to lower fatty acid production.

These results are different from the mathematical prediction given by Ranganathan et al. (Metab Eng. 2012. 14:687-704) in which down-regulation of PGM will lead to more than 20% improvement in fatty acid production. The observations also suggested that the mathematical model needs to be refined to better fit the current experimental results.

Showing similar trends are the fatty acids/glucose yield (Figure 1B). At 48 hours, the fatty acids/glucose yield of the parent strain ML103(pXZ18) is 8% higher than that of the ML103 *ΔiPGM* (pXZ18Z) mutant strain whereas the parent strain ML103(pXZ18) is 14% higher than that of the ML103 *ΔiPGM* (pXZ18Z) mutant strain. These yield data also suggest that down-regulation of PGM will not improve the fatty acid production performance of the strain.

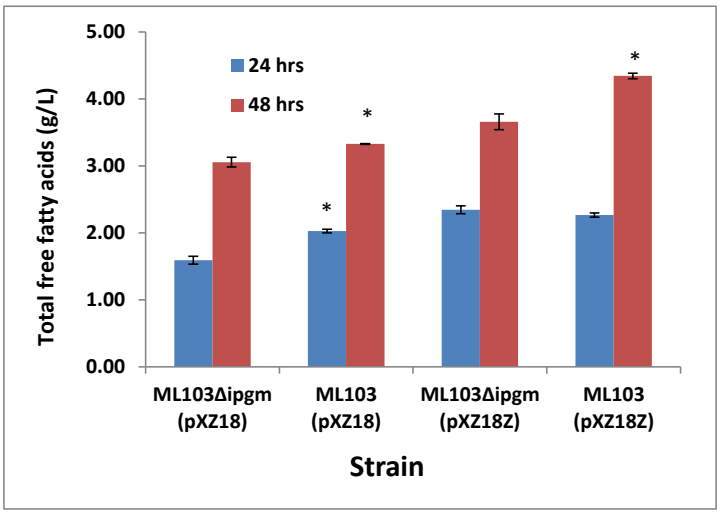

Figure 1B Comparison of fatty acid to glucose yield between parent strain and Δipgm mutant strain. *: showing statistically significant difference between two strains - T-test ($p<0.05$).

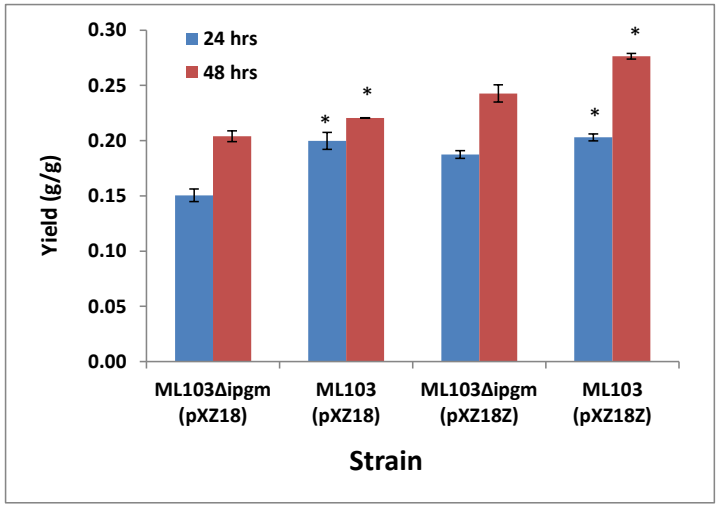

Figure 1B Comparison of fatty acid to glucose yield between parent strain and Δipgm mutant strain. *: showing statistically significant difference between two strains - T-test ($p<0.05$).

1B) Construction of strains carrying both short and long chain acyl-ACP thioesterases to improve shorter medium chain fatty acid production

To demonstrate the feedback inhibition relief concept, we used a previously constructed host strain *E. coli* strain, ML191 (MG1655, ***ΔfadDΔpfkA***), for short-chain fatty acid production. Three

plasmid systems were used in this work. One plasmid, pXZcp88, carries an acyl-ACP thioesterase specific to shorter chain length acyl-ACP. The expression of this thioesterase is under the control of an IPTG inducible *trc* promoter system. The second plasmid, pDHC29, is an expression vector which will serve as a control. The third plasmid, pDHC29-18 carries an acyl-ACP thioesterase specific to longer chain length acyl-ACP using pDHC29 as the cloning vector. The expression of this thioesterase is under the control of an IPTG inducible *lac* promoter system. The results of this system are shown in Figure 2 and Tables 1a and 1b.

Table 1a: Percentage improvement of octanoic acid production at various inducer concentrations due to the presence of the longer chain length acyl-ACP thioesterase

	Octanoic acid (g/L) IPTG @				
Strain name	100 µM	200 µM	300µM	500 µM	1000 µM
24 hr					
ML191::pXZcp88, pDHC29	ND	0.155	0.247	0.288	0.300
ML191::pXZcp88, pDHC29-18	0.276	0.383	0.445	0.443	0.479
% improvement	-	147	80	54	59
48 hr					
ML191::pXZcp88, pDHC29	0.329	0.706	0.783	0.904	0.950
ML191::pXZcp88, pDHC29-18	0.761	0.945	1.168	1.051	1.087
% improvement	131	34	49	16	14

ML191::pXZcp88, pDHC29 = *ΔfadD ΔpfkA short chain acyl-ACP thioesterase*$^+$
ML191::pXZcp88, pDHC29-18 = *ΔfadD ΔpfkA short chain acyl-ACP thioesterase*$^+$ *long chain acyl-ACP thioesterase*$^+$

Table 1b: Percentage improvement of octanoic acid yield (g of octanoic acid produced per g of glucose consumed) at various inducer concentrations due to the presence of the longer chain length acyl-ACP thioesterase

	Yield (g of octanoic acid produced per g of glucose consumed)) IPTG @				
Strain name	100 µM	200 µM	300 µM	500 µM	1000 µM
24 hr					
ML191::pXZcp88, pDHC29		0.046	0.077	0.106	0.095
ML191::pXZcp88, pDHC29-18		0.143	0.128	0.159	0.142
% improvement (yield)	-	212	67	50	49
48 hr					
ML191::pXZcp88, pDHC29	0.031	0.069	0.083	0.094	0.096
ML191::pXZcp88, pDHC29-18	0.109	0.121	0.147	0.138	0.145
% improvement (yield)	250	73	78	46	52

ML191::pXZcp88, pDHC29 = *ΔfadD ΔpfkA short chain acyl-ACP thioesterase*$^+$
ML191::pXZcp88, pDHC29-18 = *ΔfadD ΔpfkA short chain acyl-ACP thioesterase*$^+$ *long chain acyl-ACP thioesterase*$^+$

The strain carrying the longer chain length specific acyl-ACP thioesterase, ML191::pXZcp88, pDHC29-18 (ΔfadD ΔpfkA *short chain acyl-ACP thioesterase*$^+$ *long chain acyl-ACP thioesterase*$^+$), consistently outperforms the strain without, ML191::pXZcp88, pDHC29 (*ΔfadD ΔpfkA short chain acyl-ACP thioesterase*$^+$) at both time points (24 and 48 h) and at all IPTG concentrations in term of octanoic production. At an IPTG concentration of 300 µM, the production of octanoic acid (C8) is the highest among all conditions when the cells carry a longer-chain fatty acyl-ACP thioesterase, showing 49% improvement over the cells without the long-chain fatty acyl-ACP thioesterase (Figure 2 and Table 1a).

In addition, the strain carrying the longer chain length specific acyl-ACP thioesterase, ML191::pXZcp88, pDHC29-18 (*ΔfadD ΔpfkA short chain acyl-ACP thioesterase*$^+$ *long chain acyl-ACP thioesterase*$^+$), consistently gives a higher octanoic yield (g of octanoic acid produced per g of

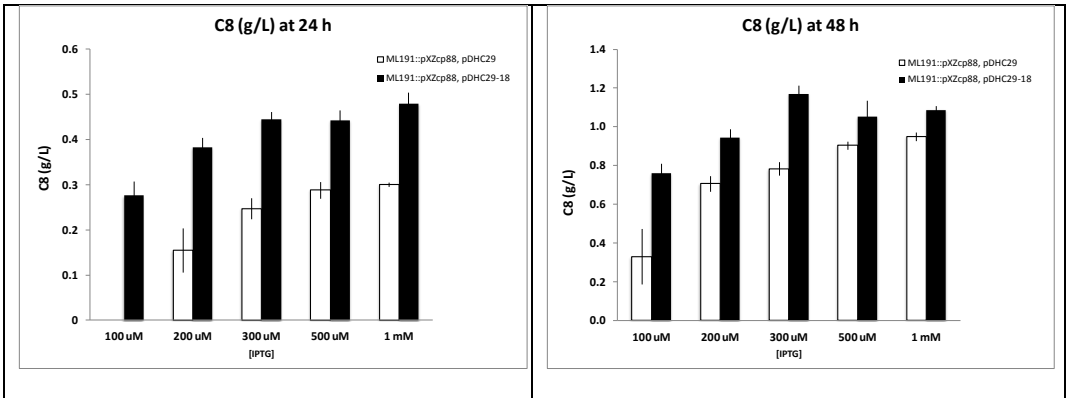

Figure 2. Comparison of the accumulation of C8 by two strain; ML191::pXZcp88, pDHC29 (*ΔfadD ΔpfkA short chain acyl-ACP thioesterase*$^+$) and ML191::pXZcp88, pDHC29-18 (*ΔfadD ΔpfkA short chain acyl-ACP thioesterase*$^+$ *long chain acyl-ACP thioesterase*$^+$) at 24 and 48 hours.

glucose consumed) than strain ML191::pXZcp88, pDHC29 (Δ*fadD* Δ*pfkA short chain acyl-ACP thioesterase*$^{+}$) at both time points (24 and 48 h) and at all IPTG concentrations (table 1b).

In summary, we have observed an increase in short chain length fatty acid, octanoic aicd, production and yield by introducing a longer chain length specific acyl-ACP thioesterase.

1C) Characterization of the impact of octanoic acid on the cell membrane and the resulting response in membrane characteristics

In the previous year we reported that short-term challenge with octanoic acid induced significant changes in membrane polarization (representative of fluidity) and leakage of Mg^{2+} (representative of membrane integrity). Additionally, we reported that the magnitude of these changes with 30mM C8 was larger than that observed for 1 wt% (~150mM) ethanol, a compound which has been well-characterized for its detrimental impact on cell membranes. Therefore, we concluded that octanoic acid has a more potent impact on *E. coli* cell membranes than ethanol.

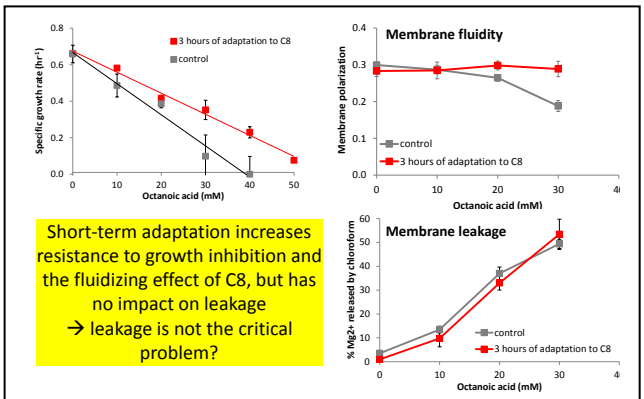

Figure 3 Effect of octanoic acid on cell membranes

We now report that when given a short period (3 hours) of adaptation to octanoic acid, resistance to growth inhibition and the membrane fluidization effect is increased (Fig 3). However, the sensitivity to membrane leakage is not changed (Fig 3). Since the increased growth resistance is accompanied by a change in membrane fluidity, but not a change in leakage, we have tentatively concluded that the leakage is not the major cause of the growth inhibition.

In order to identify changes in membrane properties that enabled resistance of the fluidization effect, we studied the membrane composition after 3 hours of growth with various doses of carboxylic acid. By quantifying C14:0, C16:1, C16:0, C17cyc, C18:1 and C18:0, we are able to calculate the saturated:unsaturated fatty acid ratio and also the average lipid chain length of these lipids. As shown in (Fig 4), both of these metrics increased in response to increasing concentration of octanoic acid; this is consistent with the trends observed in the yeast analysis (Project T2.2B). Thus, it is possible that the shift to longer, more saturated lipids enables resistance to the fluidizing effect. This is information that could be used to design engineering strategies for further increases in tolerance. We also measured the cell surface hydrophobicity based on the partitioning of cells between polar and non-polar phases. Prior to adaptation to octanoic acid, cells have a % microbial adhesion to hydrocarbons (MATH) of 22%, but this decreases to 5% during adaptation. Thus, there are many possible changes in the cell surface and membrane that could be responsible for the increased resistance and could be informative regarding the mechanisms of inhibition and resistance.

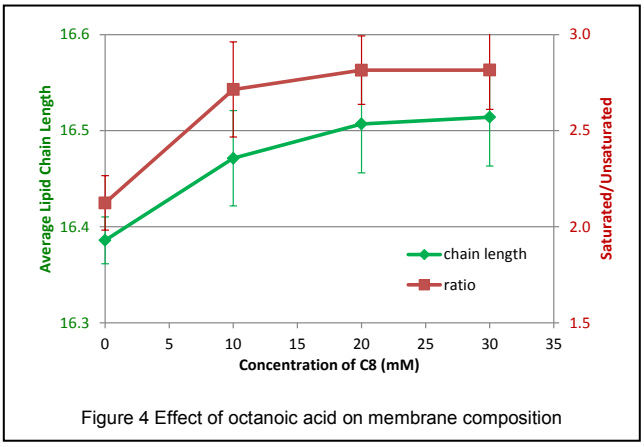

Figure 4 Effect of octanoic acid on membrane composition

The results described above used standard lab strain MG1655 with exogenously supplied octanoic acid. We have also characterized the membrane polarization and integrity of production strain ML103+pXZ18Z as a function of overall fatty acid titer (Fig 5). Consistent with the adaptation studies, it appears that the cells are able to transiently compensate for the fluidizing effect and thus membrane fluidity remains relatively unchanged throughout the course of the fermentation. Also consistent with the adaptation studies, Mg^{2+} leakage increased in accordance with the fatty acid titer.

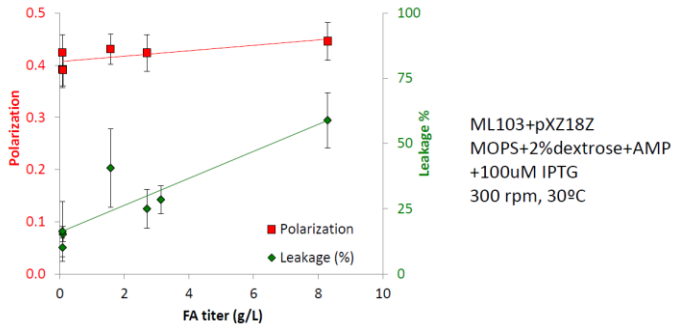

Figure 5 Changes in membrane properties during fatty acid production

Other Relevant Work: n/a

Plans for the Next Year

1 Carboxylic acids

The plans for the next years are very similar to that of project 1A. Specifically, the focus will on producing shorter chain carboxylic acids with high purity and high titer. We will be designing and performing characterization experiments to study the fatty acid producing strains developed in the *E. coli* strain construction project (1A). Furthermore, we will study the effect of various key operating conditions on strain performance.

Expected Milestones and Deliverables

The deliverables for the coming year will be quantified assessment of the performance of the *E. coli* strains plasmids and acyl-ACP thioesterase developed in Project 1A and 2A under different culture conditions. In addition, results from these characterization studies will guide the design and construction of second-generation fatty acid production systems with improved performance.

Member Company Benefits

The knowledge and constructs (plasmids and strains) being developed in this project will be useful to member companies. The knowledge leading to the possibility of producing medium to short chain carboxylic acid at high purity and high titer will have many other potential applications.

Commercialization / Technology Transfer

We are actively engaging with Center members and other companies, including start-ups, for potential licensing and commercialization of the carboxylic acid production technologies (1 US patent application, 1 PCT application and 2 provisional patent applications).

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T2.2B - Strain Characterization and Optimization in *S. cerevisiae*

Thrust: Research Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Nancy A. Da Silva	**02/14/2013**	**03/01/2012 to 02/28/2013**

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Statement of Project Goals

The goals of the work are to characterize the *Saccharomyces cerevisiae* strains (and other yeast species) under various operating conditions and to further optimize their performance for high level synthesis of carboxylic acids and pyrones.

Project's Role in Center's Strategic Plan

The goal is to characterize microbial strains for the production of two test bed chemicals, carboxylic acids and pyrones. These two test beds provide opportunities to integrate all three research thrusts. Genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the products from Thrust 2, short chain fatty acids and pyrones will serve as precursors for the synthesis of α-olefins, dienes, and other compounds by Thrust 3, the Chemical Catalysis group.

Fundamental Barriers and Methodologies

Strain characterization requires methods to analyze both the amounts and identities of the fatty acids (FAs) and related products. Further optimization requires methods to rapidly assess the effects of genetic and environmental changes. We are thus developing the needed product assays and reporter gene approaches for strain assessment. To predict strategies for increasing the performance of the strains, we will interact closely with the Omics (Project 3B), Flux Analysis (Project 4B), and Bioinformatics (Project 5B) researchers.

Achievements

During the first four years of the Center, our efforts focused on strain construction (Project 1B) and characterization including the development of required methods and strategies. During the fifth year, we have made significant progress on the characterization of our strains. We have (1)

characterized growth conditions and fatty acid/pyrone production in *Yarrowia lipolytica (Yl)*, (2) evaluated the *S. cerevisiae* strains expressing the heterologous FAS systems for activity and fatty acid synthesis, including short chain fatty acid (FA) synthesis using TEs from Thrust 1, (3) evaluated strains engineered for increased fatty acid synthesis, (4) evaluated strains for the high-level synthesis of pyrones.

(1) Characterization of growth conditions and fatty acid/pyrone production in *Yarrowia lipolytica (Yl)*

We have characterized the growth conditions for carbon and nitrogen limitation and characterized lipid production in *Yarrowia lipolytica (Yl)*. Conditions under which *Yl* becomes carbon limited are: 0.17% YNB, 0.5% ammonium sulfate, and 1% dextrose; conditions for nitrogen limitation are: 0.17% YNB, 0.0625% ammonium sulfate, 0.5% sodium sulfate, and 2% dextrose. Lipid production was tested in cells in log, late log, early stationary, and late stationary. In late stationary, nitrogen-limited cultures produce four-fold greater lipid than carbon-limited cells. Total lipid content was evaluated by the UC Davis Metabolomics Core using isopropanol and cyclohexane extraction (method of Smedes, 1999), methylation with sodium methoxide and methanoic HCl followed by GC-MS. The major fatty acid was 18:1, with second highest C18:2 in both carbon- and nitrogen-limited growth. This reflected greater than 20% fatty acid per gram dry weight in the nitrogen-limited sample.

For the production of pyrones in *Yl*, the gene for *Gerbera hybrida* pyrone synthase was expressed from the pJY3763 shuttle plasmid under the *YlTEF* promoter. Supernatants of stationary phase cells were measured by HPLC. Initial tri-acetic acid lactone concentrations were quite low (16 mg/l), but this was in the absence of strain or enzyme engineering. Expression levels of a tagged version of the gene are currently being tested in order to determine if the protein is expressed and stable.

The gene for mitochondrial glycerol-3-phosphate dehydrogenase was deleted and the resulting *gut2Δ* mutant was characterized. The glycerol-3-phosphate dehydrogenase gene was deleted using *URA3,* flanked upstream and downstream by 1000bp of *GUT2* flanking sequence, selected on ura- medium, screened on glycerol for no growth, and confirmed by PCR. This strain was grown under nitrogen-limiting conditions and unsaturated lipids were evaluated using the phospho-vanillin assay. The level of these lipids in the *gut2Δ* strain was 250% of wildtype. This strain will be tested to determine whether it also has elevated expression of testbed products derived from two and three carbon modules.

(2) Characterization of heterologous FAS strains for fatty acid synthesis in *S. cerevisiae*

The activity of the mammalian FAS (mFAS) system expressed in *S. cerevisiae* was previously confirmed both *in vitro* and *in vivo*. Expression of mFAS *in vivo* complemented a yeast *FAS2* knockout and allowed growth of the yeast in the absence of exogenous fatty acid supplements. The *in vitro* activity of the *E. coli* FAS enzymes (expressed individually in yeast) was also previously confirmed.

Over the past year the integration of the eight required *E. coli* genes into a single yeast strain was achieved using an optimized integration method. For the evaluation of the *in vivo* activity of the heterologous system, the strain carrying the type II FAS system was transformed with a selection of thioesterases (TEs) from other hosts. These TEs have been show in the literature to have different chain-length cleavage preferences. Two assays were used to determine the ability of the heterologous FAS to produce fatty acids in yeast. The first was a growth

complementation assay similar to that used for the mFAS. The native yeast FAS2 gene was deleted in a strain carrying the full *E. coli* FAS system and tested for growth in the absence of FA supplementation. The strain was able to grow on both plates and in liquid culture using the heterologous FAS system. The second looked at FA production in yeast strains carrying both the native FAS and the new FASII system. Intracellular and extracellular FA production was measured using GC-MS for C_{16} and C_{18}, and GC-GC-MS for shorter-chain FAs. Total intracellular FA results are shown in Figure 1; in this experiment, three TEs were evaluated and fatty acid levels were significantly higher than with the yeast FAS alone.

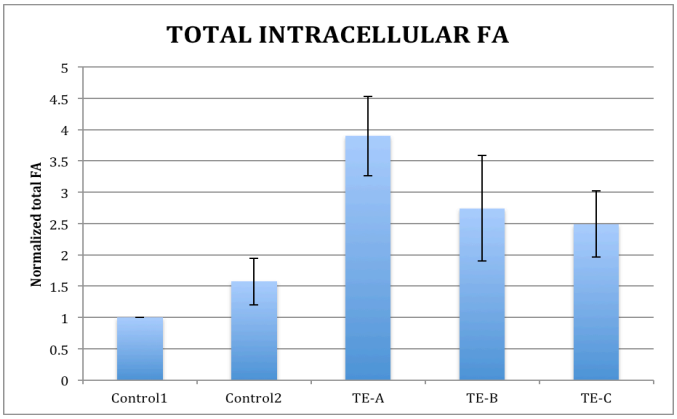

Figure 1: Intracellular fatty acid levels for strains with the type II FAS integrated, and thioesterases on a plasmid. Control1 and control2 were transformed with a blank plasmid. Cells were grown in SDC(A) for 20h. Data from two independent runs.

For the synthesis of short chain fatty acids, we have combined novel thioesterases from Thrust I and two TEs from the literature with our new heterologous yeast systems. The activity of the TEs produced in yeast were confirmed using *in vitro* assays (with highest activity seen at shorter carbon lengths for the enzymes assayed). The TEs with the highest activity have been transformed into the yeast strain carrying mFAS lacking the TE domain. We have also created two mutant mFAS constructs with a short chain thioesterase domain fused directly to the mFAS via a linker. These short chain TE domains replaces the native TE domain and allow the direct shuttling of acyl-CoA substrates from the adjacent ACP domain. Studies using the GC-GC-MS showed substantial improvement in short chain fatty acid production over controls. A 32-fold increase, 21-fold increase, and 19-fold increase was observed for C_8 levels, C_{10} levels and total short chain fatty acid levels over controls, respectively. Additionally, for the first time we have observed the *in vivo* production of C_6 using these two mutant mFAS constructs.

(3) Evaluation of strains engineered for increased fatty acid synthesis

To engineer strains for increased production of fatty acids and related compounds, we have knocked out specific regulatory and pathway genes, and upregulated genes for the synthesis of important precursors (e.g., acetyl-CoA, malonyl-CoA) (Project 1B). One approach to increase the pool of acetyl-CoA has utilized a strategy from the oleaginous yeasts known to accumulate fatty acids.

Another major focus has been the control and down-regulation of the native yeast fatty acid synthase. The native FAS is the primary consumer of both acetyl-CoA and malonyl-CoA. The overall strategy is to utilize the native FAS for initial growth, then down-regulate this FAS so that the acetyl-CoA and malonyl-CoA pools are available to the heterologous FAS for short chain fatty acid synthesis. In an initial experiment, we integrated a strong glycolytic promoter in front of the *FAS2* gene, which removes native transcription regulation. This strain had a 25% reduction in fatty acid levels in the stationary phase compared to the BY4741 control

(Figure 2). This observation demonstrates the ability to control native fatty acid levels by controlling the native FAS expression via manipulation of the *FAS2* promoter region. Current experiments are assessing various constitutive and inducible promoters for native FAS synthesis, and *in vivo* fatty acid production will be determined in early exponential, late exponential and stationary phases with the GC-MS.

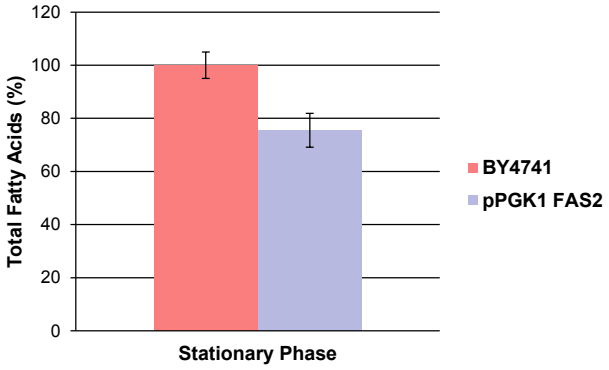

Figure 2: Replacing the yeast *FAS2* promoter with the *PGK1* promoter reduces native yeast fatty acid (C_{16}, C_{18}) production.

Fatty acid synthesis is initiated when an acetyl moiety from acetyl coenzyme A is transferred to the thiol group of the phosphopantetheine arm of the acyl carrier protein by the phosphopantetheine transferase (PPT). mFAS lacks a PPT domain, thus requiring an external PPT activator. To address this, we have evaluated three different PPTs (2 bacterial, 1 eucaryotic) for efficient activation of the mFAS. The results from an initial study have shown efficient activation for two of the three PPTs (Figure 3).

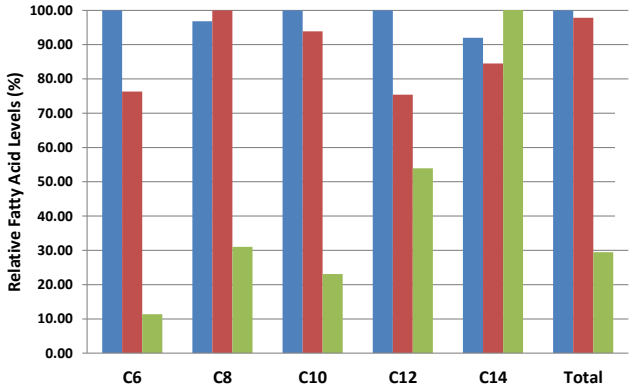

Figure 3: Relative *in vivo* fatty acid levels using three different phosphopantetheinyl transfereases.

(4) Evaluation of strains engineered for the synthesis of pyrones

To increase pyrone synthesis, several strategies have been pursued including the assessment of new enzyme variants (developed by the Noel lab, Thrust 1), preventing proteolysis of the synthase, increasing 2-PS expression levels (via copy number and promoter choice), engineering the strain for increased precursor synthesis. The triacetic acid lactone (TAL) produced is measured in the culture medium using HPLC.

Forty-one 2-PS mutants from the Noel lab have been cloned into pXP vectors and expressed in our yeast strain. TAL levels up to 175% over widltype were observed for the twenty-three variants tested to-date (Figure 5). Additional mutants, including combinations of the more promising ones, are currently being screened.

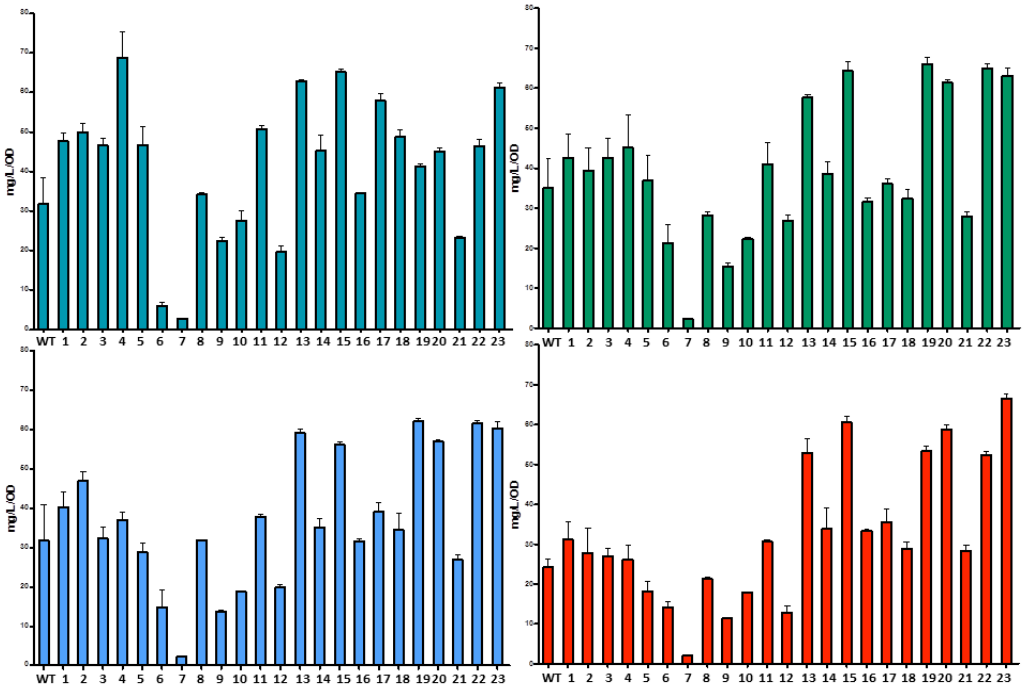

Figure 5: Pyrone levels in protease deficient strains using all 2-PS mutants tested to-date (mutants #1-23) as compared against the wildtype (WT) 2-PS.

In addition, strains are being engineered for higher TAL synthesis. Precursor availability was improved by identifying bottlenecks via computational modeling in addition to manipulations identified by recent publications. Use of OptKnock in the COBRA 2.0 Toolkit indicated that strains inhibited in both fatty acid synthesis and pyruvate carboxylase activity, as well as removing glycogen and glycerol biosynthesis should increase TAL levels. Testing single gene deletion strains resulted in nearly 3-fold higher TAL productivities (Figure 6).

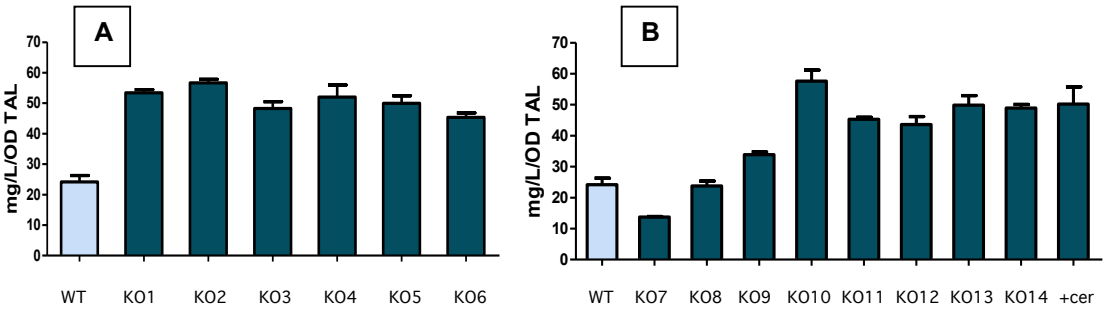

Figure 6: Pyrone levels for strains with single gene knockouts or cerulenin addition selected via (A) OptKnock and (B) Literature review. The wildtype 2-PS was used in all cases.

Current efforts are aimed at combining multiple single gene deletions for improved productivity. We have also combined our strain and enzyme engineering (Thrust 1) strategies. A strain with 2 protease and 2 pathway gene knockouts was evaluated using 3 promising mutant synthases from the Noel Lab. This yielded a TAL titer of 1.74 g/L and 37% of the theoretical yield (Figure 7). Further strain and synthase manipulations should result in even more robust systems for the production of triacetic acid lactone in *S. cerevisiae*. We are currently providing culture broth to Thrust 3 for their catalysis work.

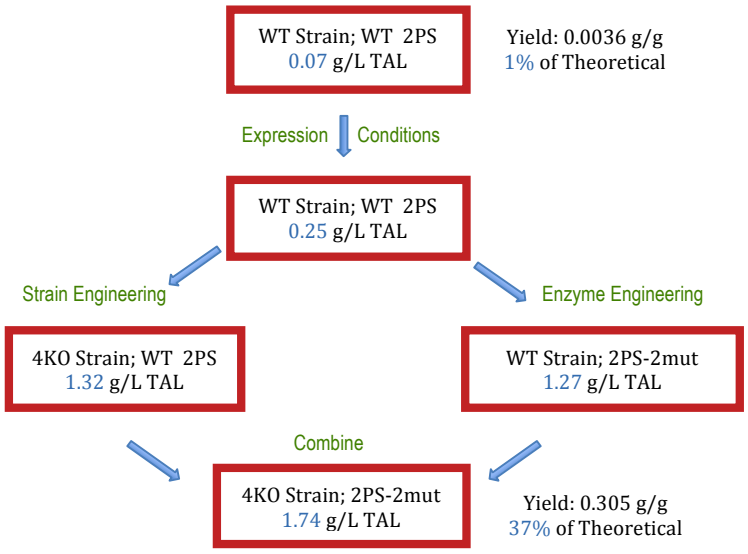

Figure 7: Increases in TAL production via strain engineering, enzyme engineering, or a combination of both. An increase of 1% to 37% of theoretical yield has been obtained.

Ongoing work focuses on combining the most promising strategies and assessing further routes for improvement. We are also evaluating strategies for obtaining similar yields in yeast minimal medium, critical for the flux experiments and more relevant for industrial applications.

Other Relevant Work

Similar experimental work is being conducted within CBiRC using *E. coli* as the model microbial system. In combination, the research will evaluate three promising microbial systems for the synthesis of the precursor compounds required for the Center's goals. Other methodologies utilized within CBiRC (e.g., DNA microarrays, proteomics, and flux analysis) will also provide key information to guide future strain development and characterization.

Plans for the Next Year

Characterize and optimize strains for increased titer and yield of short chain fatty acids and pyrones:

Characterize the strains developed in Project 1B: Measure fatty acid and pyrone levels; evaluate removal of pathway bottlenecks.

Combine information from Omics, Flux, and Bioinformatics groups with targeted characterization studies to guide further strain development and optimization.

Characterize strains expressing new enzymes from Thrust 1

Expected Milestones and Deliverables

Tools for characterization of strains producing carboxylic acids and pyrones

Determination of FAS/TE combination for synthesis of fatty acids of specified chain length

Identification of key bottlenecks in the synthesis of fatty acids and polyketides

Member Company Benefits

The benefits for the Center's industry members are the characterization and further optimization of strains for the high-level synthesis of carboxylic acids, pyrones, and other desired compounds. The methods developed and the integration with Omics, Flux Analysis, and Bioinformatics efforts will demonstrate the effectiveness of the metabolic engineering optimization cycle.

Commercialization / Technology Transfer

Opportunities for technology transfer and commercialization are expected from the work.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.3A** - Omics Experiments in *E. coli*

Thrust: Research Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Ramon Gonzalez	**02/14/13**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Ramon Gonzalez, Rice University
Other Faculty: Laura Jarboe and Julie Dickerson, Iowa State University
Graduate Students: Maria Rodriguez-Moya, Rice University; Liam Royce, Jesse Walsh, and Erin Boggess, Iowa State University

Statement of Project Goals

This project aims to use functional genomics tools to: i) identify the metabolic response of *E. coli* to inhibitory concentrations of short chain fatty acids (SCFA) and ii) assess the metabolic changes resulting from the engineering of pathways for the production of SCFA in *E. coli*. System-wide characterization of gene and protein expression will be performed by DNA microarrays and 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) combined with Mass Spectroscopy. The outcomes of both objectives will support the engineering of strains able to produce and tolerate high levels of fatty acids.

Project's Role in Center's Strategic Plan

The results from this project will directly contribute to both test beds proposed in the Center's strategic plan, namely the production of carboxylic acids and dienes. The functional genomic analysis of strains producing specific products in each of these test beds will contribute to the elucidation of the underlying mechanisms mediating their metabolic performance. These results, in turn, will guide engineering efforts to construct high-producing and high-tolerant strains. The establishment of this systems biology based approach would be of great assistance in the design of other biocatalysts.

Fundamental Barriers and Methodologies

This project could be limited, in general, by the ability to integrate functional genomics approaches into the traditional strain development/metabolic engineering cycle.
Data analysis and interpretation of combined functional genomics studies could also be a barrier.
New approaches and techniques currently under development in the "Bioinformatics" projects will be of tremendous help in overcoming the above barriers.

Achievements

Role of differentially expressed proteins

The individual role of the proteins that were differentially expressed in the Proteomic Analysis presented in the previous report (2012) has been studied in further detail. Initially, the differences in maximum OD (at 4 hours) of individual knockout and overexpression (in high copy vector) mutants corresponding to the differentially expressed proteins (Figure 1) were analyzed in order to determine if any particular protein had a relevant response to octanoic acid in the medium. For example, knockout strains that exhibited a significant difference in growth with respect to the wild-type would suggest a strong influence of that particular gene/protein on tolerance to fatty acids. The difference of maximum OD between cultures with 0mM and 15mM octanoic acid was calculated for each mutant strain in order to determine if the mutation is beneficial (smaller difference) or detrimental (larger difference) to *E. coli* growth on fatty acids.

Difference in Maximum OD = (Max OD @ 0mM C8) – (Max OD @ 15mM C8)

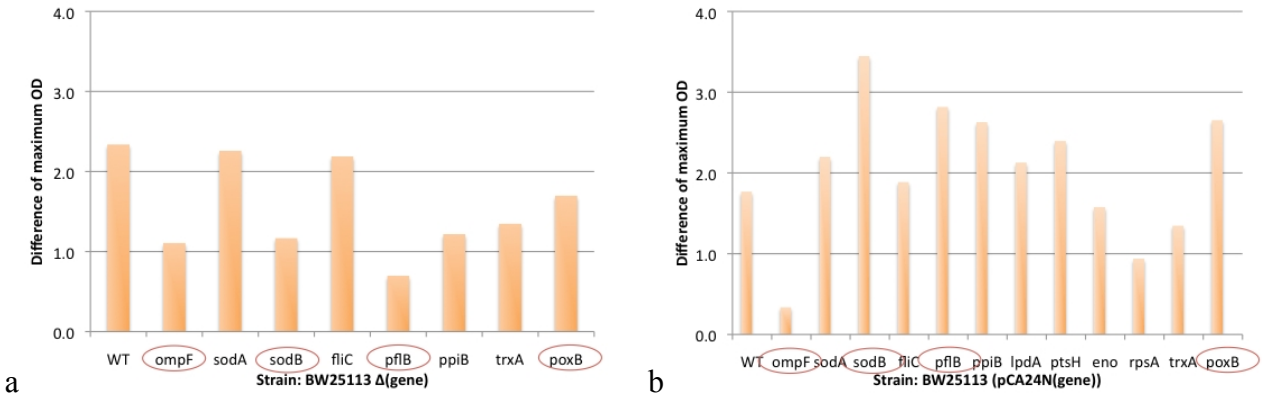

Figure 1. The difference of maximum growth for each (a) knock-out and (b) over-expression mutant was calculated. The control used for the knock-out mutants was wild-type BW25113 and for the over-expression mutants, BW25113 pCA24N (blank). Four proteins were selected for further analysis.

Out of the 12 proteins that were analyzed, four proteins showed a beneficial difference when knocked-out and a detrimental difference when over-expressed in a high copy vector. These four proteins were selected and studied in more detail by expressing them in low- and medium-copy vectors, using different concentrations of inducer (Figure 2).

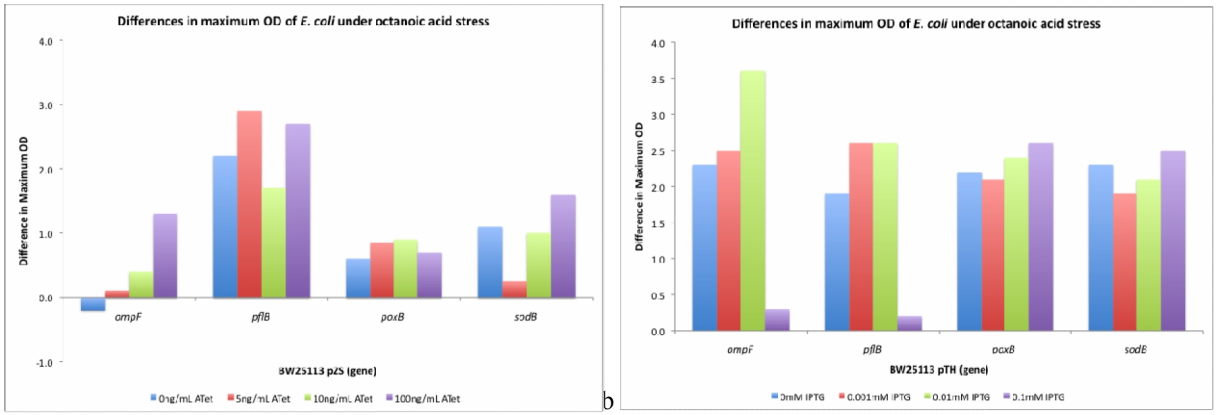

Figure 2. Differences in maximum OD of *Escherichia coli* under octanoic acid stress. The effects of over-expressing four proteins (OmpF, PflB, PoxB, and SodB) in (a) low- and (b) medium-copy vectors was analyzed. Outer membrane porin F was highlighted as a promising target for improving tolerance of *E. coli* to short-chain fatty acids.

Eight mutant strains were constructed (by Phusion reaction) from the four proteins that were selected in the analysis mentioned above: four low-copy mutants (BW25113pZS*ompF*, BW25113pZS*pflB*, BW25113pZS*poxB,* and BW25113pZS*sodB*) and four medium-copy mutants (BW25113pTH*ompF*, BW25113pTH*pflB*, BW25113pTH*poxB,* and BW25113pTH*sodB*). Each strain was analyzed using different concentrations of inducers for gene expression (anhydrotetracycline for pZS vector, and IPTG for pTrcHis2A (pTH) vector).

Proteins involved in redox stresses (SodB), as well as proteins involved in carbon metabolism (PflB and PoxB) are also of interest. Of particular interest in this analysis is the dramatic effect that the deletion of *ompF* and its expression in a low-copy plasmid have over cell growth. Different levels of protein expression, obtained from different copy vectors and different inducer concentration, revealed that the leaky expression of OmpF in the low-copy plasmid pZS is beneficial for growth of *E. coli* in the presence of octanoic acid. This protein will be the main focus of future studies in the Omics project. This outer membrane porin has been proposed as a participant in transport of longer-chain fatty acids across the cell membrane, and has been reported as having an effect on multidrug tolerance (Antimicrobial Agents and Chemotherapy, 2009, 53:4944-4948). Additional studies on the effects of different levels of expression of this and other outer membrane porins could provide valuable information about fatty acid transport across the membrane and about their toxicity mechanisms. A more thorough study of membrane porins will be conducted.

Other Relevant Work

Preparation of manuscripts for publication:

Cintolesi, A., Rodriguez-Moya, M., and Gonzalez, R. (2013). Disorders of fatty acid oxidation. *WIREs System Biology and Medicine.* (MS in Review).

Royce, L., Ping, L., Stebbins, M., Hanson, B., and Jarboe, L. (2013). The Damaging Effects of Short Chain Fatty Acids on *Escherichia coli* Membranes. *J. of Biological Chemistry.* (MS in Review).

Plans for the Next Five Years

Further omic studies will be performed to compare the effects of octanoic acid to other fatty acids or different chain lengths.

Strains will be constructed according to the gene deletions and over-expressions that are selected as most favorable and combining them with modifications from fatty acid-producing strains that have been developed in the strain optimization and construction project.

Expected Milestones and Deliverables

For next year, we expect to:
1. Further characterization of protein roles is expected, in particular, studying variations in expression of outer membrane porins, since they are assumed to play a key role in tolerance of *E. coli* to octanoic acid.
2.Study the effect of variations in expression of the differentially expressed proteins on specific growth rate and determine which of these mutations are beneficial/detrimental to cell growth rate.
3. Use high-throughput method to quickly transform and screen for carboxylic acid tolerance.

Member Company Benefits

The understanding of the response of *E. coli* to inhibitory concentrations of SCFAs and its harnessing to obtain strains that are tolerant to high concentrations of fatty acids is expected to generate significant intellectual property, which in turn will benefit member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.3B** - Omics Experiments in *S. cerevisiae*

Thrust: Research Thrust 2 – Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Laura R. Jarboe	**2/14/13**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Laura Jarboe, Iowa State University

Other Faculty: Suzanne Sandmeyer and Nancy DaSilva, University of California, Irvine

Postdoctoral Researchers: Uyen Phuong Tran, Ivan Chang, University of California, Irvine

Graduate Students: Ping Liu, Iowa State University; James Yu, University of California, Irvine

Undergraduate Students: Lucas Kerns, Iowa State University

Statement of Project Goals

The goals of this project are two-fold: (1) analyze strains that have increased production of the target compound, in order to formulate strategies for additional strain improvement and (2) analyze our biocatalyst during challenge with an inhibitory concentration of the target compound, in order to engineer the strain or growth condition to alleviate this inhibition. In the work described here, short-chain carboxylic acids are the target product.

Project's Role in Center's Strategic Plan

This project serves the central CBiRC Strategic Plan by contributing to the carboxylic acid test bed and will lead to the development of a standard method for strain optimization and characterization for future test beds. Specifically, this project will aid in both the understanding of the fatty-acid biocatalytic machinery and in the design of efficient biocatalyst systems and contributes to the T2 and T3 critical milestones.

Fundamental Barriers and Methodologies

This project is limited, relative to the *E. coli* project, by the decreased availability of pathway and annotation data of *S. cerevisiae*.

The goal to perform transcriptome analysis and flux analysis in parallel leads to increased stringency for experimental design, given the experimental constraints for flux analysis.

Simultaneous analysis of transcriptome and fluxome data will present technical barriers in terms of data storage and visualization.

Simultaneous interpretation of transcriptome and fluxome data is relatively new to this area.

Achievements

(1) Compare strains with increased production of the target compound to our host strain

While construction of our carboxylic acid-producing *S. cerevisiae* continues, omics analysis has focused on the oleaginous yeast *Yarrowia Iipolytica* (YI). Note that YI is able to accumulate lipids at up to 50% of the dry mass. Our team is developing YI as a system for expression of pathways sharing precursors with fatty acids. However, Yl has not been as thoroughly characterized as the biocatalyst *S. cerevisiae* (Sc). We are increasing this characterization through the use of current technologies that allow collection of information-intensive transcriptomic, proteomic, metabolomic, and fluxomic data in both Sc and YI. These data can be used to model *Sc* metabolism, but also provide a scaffold from which a *Yl* model is projected. Flux distribution maps (generated through CellDesigner) predicted through each model highlight the differences between the model systems.

Toward integration of heterogeneous omics data and successful projection of the Sc model onto the Yl model, the current assembly of the *Yl* genome is being updated to allow mapping of Sc orthologs and Yl transcripts, as well as a better reconstruction of the Yl genome-scale reaction network model. In addition a new computational modeling framework (denoted as the hybrid kinetics-constraint model) is being developed which combines a layer of detailed kinetics describing central metabolism with an underlying genome-scale reaction network containing stoichiometric relationships based on mass balance.

An updated assembly of the Yl genome is nearing completion. This sequence assembly will be based on Illumina sequencing reads (deep, but short) and PacBio RS sequencing (shallow, but long). The former is used to correct the low coverage reads while that latter is used to connect the short contigs. A working prototype of the hybrid kinetics-constraint model (with a kinetic core of glycolysis+PPP+FA reactions) which can be adapted to either Sc or Yl kinetics and several omics data types (TAPTAG proteomics, RNASeq transcriptomics, and 13C fluxomics) has been created using a combination of Mathematica/kMech and Matlab/COBRA Toolbox packages (Figure 1). Auxiliary supporting programs have been introduced to this modeling framework. These include automated data-mining from KEGG and BRENDA online databases to substitute best-fit values for missing kinetic parameters; gradient-descent based optimization of kinetic parameters to steady-state metabolic concentration profiles; and iterative convergence optimization of the boundary reactions between the kinetics model and the genome-scale network model based on the gradient projection method for nonlinear programming.

(2) Analyze our biocatalyst during challenge with the target product

We previously described our transcriptome analysis of the *S. cerevisiae* laboratory strain BY4741 during challenge with 0.3 mM C8 at pH 5.0. Note that this concentration is sufficient to inhibit growth by 25%. This analysis led to the proposition and verification that membrane integrity is damaged during exposure to carboxylic acids, as evidenced by increased leakage of Mg^{2+}. The magnitude of this leakage increased in a dose-dependent manner and also increased as carboxylic acid chain length increased, similar to the trends observed with the growth inhibition. Allowing cells to adapt to carboxylic acid exposure decreased the magnitude of this leakage and was accompanied by a change in the membrane composition. Generally speaking, this change involved a decrease in C16:1 and corresponding increase in C18:1 (oleic acid). As we reported in year 4, supplementing the media with C18:1 increases octanoic acid tolerance.

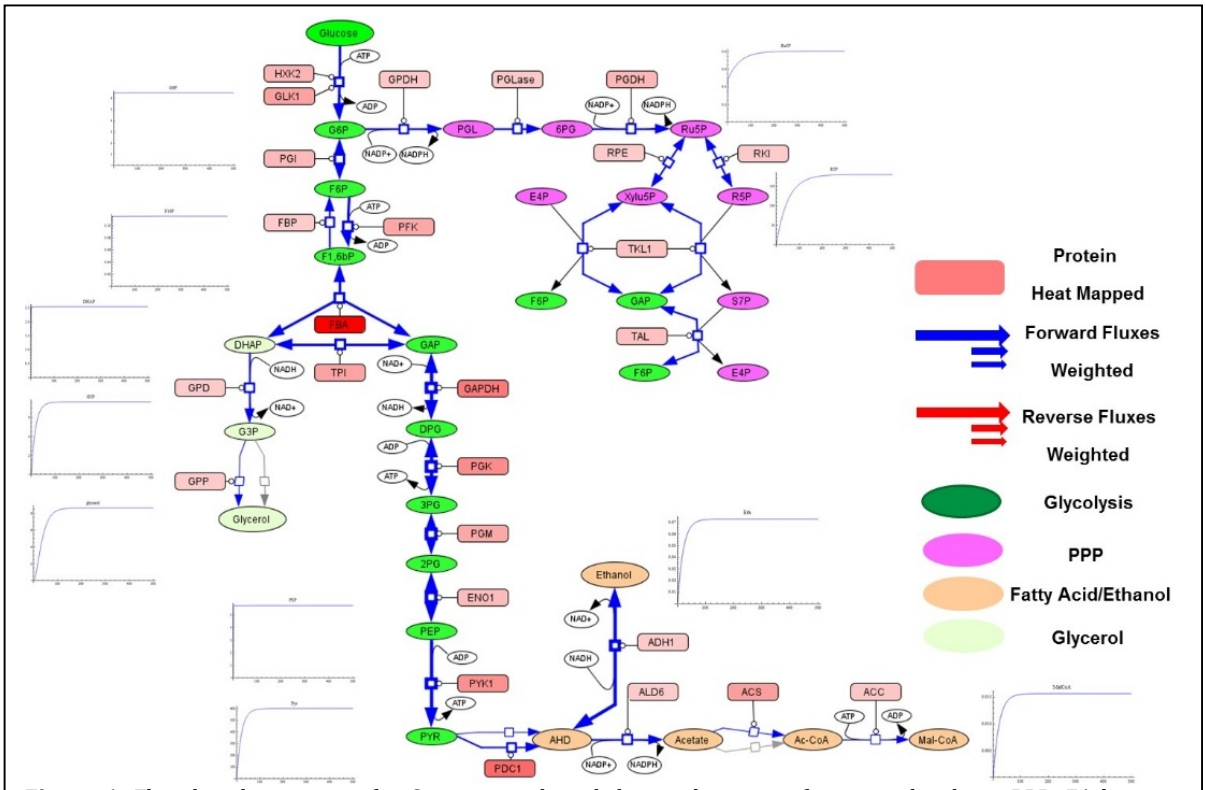

Figure 1: Flux distribution map for *S. cerevisiae* based the combination of a yeast glycolysis+PPP+FA kinetics model and the Yeast Consensus 5.22 genome-scale model, infused with 13C fluxomics and TAPTAG proteomics data. Shown next to the metabolites are time-series concentration curves produced from kinetics simulation. Legend: Edges are weighted by flux magnitude, with blue edges = forward fluxes, red = reverse fluxes. Enzyme label color intensity are weighted by concentration, with bright red = highest, and light pink = lowest. Glycolysis metabolites in green, PPP metabolites in purple, and Ethanol/FA pathway metabolites in orange.

This year we focused on understanding how this supplementation is helpful for C8 tolerance and engineering the cells for increased C18:1 content in the membrane so that costly media supplementation would not be required.

As shown in Figure 2, the increased growth resistance conferred by C18:1 supplementation is accompanied by a decrease in C8-mediated membrane leakage. Membrane composition analysis showed that the C18:1 content in the membrane during challenge with 0.5mM C8 increased from 35% (by area) in the unsupplemented condition to 55% in the C18:1-supplemented condition (*data not shown*). Thus, we concluded that the oleic acid supplementation is beneficial to carboxylic acid tolerance because the oleic acid is incorporated into the membrane and this high C18:1 membrane content helps to mitigate the membrane leakage caused by carboxylic acids.

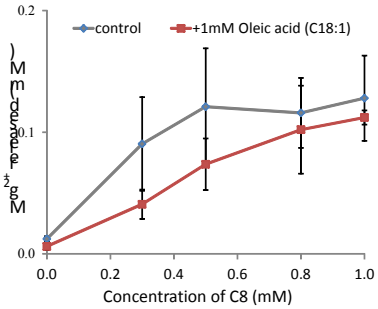

Figure 2: In addition to increasing the growth resistance to C8 (reported in Y4), supplemental oleic acid (C18:1) decreases the detrimental effect of C8 on membrane integrity.

We attempted to modify our strain to produce its own C18:1, avoiding dependence on costly media supplementation. The use of the acyl-CoA 9-desaturase (OLE1) has previously been reported to contribute to ethanol tolerance (You 2003; Kajiwara 2000), where OLE1 converts saturated fatty acids to unsaturated fatty acids. Therefore, we engineered our strain to express the TniNPVE desaturase; this enzyme was previously demonstrated to increase oleic acid content in yeast membranes (You 2003). While expression of this enzyme was able to increase oleic acid content in our cell membranes from 20% (by area) to 30% in the absence of C8 challenge, we were unable to achieve the >50% C18:1 content needed to counter the damaging effects caused by carboxylic acids. Thus, there was no increase in C8 tolerance due to expression of the TniNPVE desaturase, despite demonstrated activity of this enzyme (*data not shown*). This is presumably due to the tight metabolic control of membrane composition by the native *S. cerevisiae* regulatory systems. Media supplementation with oleic acid can circumvent this regulatory control and enable construction of a stronger membrane, but independent construction of stronger membranes without oleic acid supplementation would require characterization and engineering of the regulatory systems.

We also attempted to increase membrane integrity through expression of the *Escherichia coli* cyclopropanated fatty acyl phospholipid synthase (*cfa*). Cyclopropanated fatty acids have previously been reported to contribute to acid resistance in *E. coli* (Chang and Cronan 1999). Similar to the results observed with the TniNPVE, our recombinant expression system was successful in changing the membrane composition (C17cyc was present as more than 10% (by area) of the membrane lipids), but there was no observable increase in octanoic acid tolerance. To the best of our knowledge, this was the first successful engineering of *S. cerevisiae* to produce cyclopropanated fatty acids.

Thus, our omics analysis of *S. cerevisiae* during challenge with octanoic acid has suggested that the primary cause of growth inhibition is damage to the cell membrane. This damage can cause leakage of vital metabolites. Here we have used Mg^{2+} as an easily-measured "lost" metabolite, but it is certainly not the only one. The membrane can be made stronger, and thus the strain can be made more resistant, by increasing the oleic acid content in the membrane. However, the necessary level of oleic acid can only be reached at this time by directly supplementing the media with oleic acid. Additional engineering efforts are needed to enable the cells to reach the necessary level on their own. This work was accepted for publication by "Applied Microbiology and Biotechnology (2011 impact factor 3.425) on February 11th, 2013.

Future work could focus on (a) engineering strategies for strengthening the cell membrane or (b) identifying additional mechanisms of inhibition, by performing additional transcriptome analysis while supplementing the media with oleic acid. Note that the protective effect of oleic acid supplementation is only moderate. It enables growth in the presence of 1mM octanoic acid, a concentration which is completely inhibitory in the unsupplemented condition, but 2mM octanoic acid is sufficient to completely inhibit growth even with supplementation. Thus, there are certainly additional mechanisms of inhibition that need to be identified and addressed.

Other Relevant Work

Goal 1: Analysis of strains with increased fatty acid production.

Yazawa et al Yeast 2009 analyzed the transcriptome of *S. cerevisiae* engineered to produce polyunsaturated fatty acids (PUFA). Their transcriptome analysis led to many interesting findings, such as the fact that PUFA production is linked to the alkaline stress response. However, their analysis differed from ours in that they did not have a rigorous method for analyzing or visualizing their transcriptome data.

Goal 2: Analysis of inhibitory response

Previous researchers have looked at the growth response of yeast to fatty acid stress, but this stress was not investigated at a systems level. Instead, they screened insertion libraries or investigated specific enzymes, such as H^+-ATPase. This project differs in that we will be investigating the systems-level response to inhibition by and production of short-chain fatty acid and will be integrating this data with flux analysis.

Abbott et al FEMS Yeast Res (2007) used transcriptional analysis of the weak organic acid response to define a "generic" response during anaerobic growth. However, none of the genes in their defined generic response are perturbed in our datasets.

Plans for the Next Year

1. Continue with the comparative analysis of Sc and YI
2. Perform omics analysis on strains engineered for carboxylic acid or pyrone production
3. Integrate the *S. cerevisiae* C8 challenge data (described here) with the *S. cerevisiae* C8 challenge flux analysis data

Expected Milestones and Deliverables

Simultaneous transcriptome/flux analysis during moderate carboxylic acid challenge.

Transcriptome and flux analysis of the engineered carboxylic acid-producing and pyrone-producing strain(s) – this is a long-term goal.

Member Company Benefits

It is anticipated that this research project will generate valuable know-how and/or intellectual property for member companies. This includes a general framework for using omics analysis to identify metabolic bottlenecks and toxicity of substrate or product compounds, something that is very relevant to cellulosic ethanol and next-gen biofuels.

Commercialization / Technology Transfer

Opportunities for technology transfer and commercialization are expected from the work.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.4A -** Flux Analysis in *E. coli*

Thrust: Research Thrust 2 **-** Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Jacqueline V. Shanks	**02/21/2013**	**03/01/2012 to 02/28/2013**

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Assistant Scientist:
Jong Moon Yoon, Department of Chemical and Biological Engineering, ISU

Statement of Project Goals

The goal of the project is to construct metabolic flux maps for *E. coli*, for both the wild-type and engineered strains and under various operating conditions. The flux maps from this project will be used to guide further genetic manipulations for strain improvement.

Project's Role in Center's Strategic Plan

Metabolic flux maps are an integral part of the metabolic engineering design cycle to construct strains that produce carboxylic acids and pyrones, the intermediate biochemicals of two CBIRC test beds that require catalysis via Thrust 3 to make the α-olefins and dienes or dienoic acids, respectively. Metabolic fluxes are an important physiological characteristic, providing a global perspective of the integrated functioning between levels of transcripts, proteins, and metabolites to cellular phenotype. Metabolic flux analysis identifies potential bottlenecks in the reaction network that limit production of the target compound. These bottlenecks are then genetically engineered out in the next metabolic engineering cycle.

Fundamental Barriers and Methodologies

A key barrier in the overall goals of Thrust 2 will be to shorten the metabolic engineering cycle. Since flux plays an integral role in the metabolic engineering cycle, this means a quick turnaround time for flux analysis results to the strain construction and bioinformatics projects. Fundamental barriers for metabolic flux analysis lie in (1) the validation of the flux map, (2) in deciding the right metabolic flux analysis mapping tool for the application, and in (3) the correct basis of the metabolic flux results to integrate into the bioinformatics framework for comparison to other data sets. For validation of the flux map, the network topology and nomenclature is coordinated with the CBIRC-enhanced Ecocyc pathway database as well as using large scale isotopomer models, and CBiRC generated 'omics data.

In deciding the tradeoff in the time intensive but information rich comprehensive flux analysis versus a more high-throughput "fluxomics" method (which either only uses partial labeling information to obtain a flux map or correlates labeling information via a bioinformatics approach) an assessment of conventional MFA and fluxomics MFA, was benchmarked with comprehensive MFA, so that a design strategy can be assessed so that more strains can be characterized at the level needed.

Achievements

Integration of Experimental and Computational Approach for Strain Design

Metabolic flux map for wild type MG1655 under aerobic condition was generated. The MFA data was used to characterise the wild-type phenotype using the *i*AF1260 genome-scale model of *E. coli*. The OptForce procedure subsequently identifies engineering intervention strategies for overproduction of fatty acids of chain lengths varying from C6-C16. The intervention strategies for each same length have been summarized in the Venn diagram in Figure 1. As clearly seen from the figure, no universal engineering strategy was predicted for overproduction of all fatty acids, indicating the chain length specificity of each of the strategies.

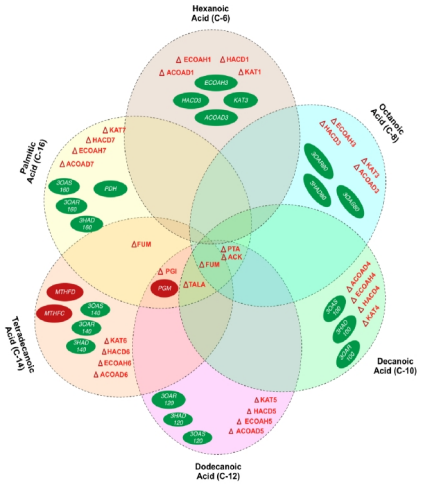

Figure 1. Venn diagram representing the shared genetic interventions predicted by OptForce for fatty acids of chain length C_6 to C_{16}.

The genetic manipulations required in *E. coli* for the overproduction of palmitate (C_{16} fatty acid) and corresponding impact on the yield are shown in Figure 2. As seen in the figure, the up-regulation of one of the elongation reactions in the C_{16} chain conjunction with a reaction removal in the β-oxidation pathway, along with redirection of the glycolytic flux leads to an increase in the yield of about 32% of the theoretical maximum. Additional deletions and knock-downs result in improving the yield close to 66% of theoretical maximum. In accordance with the OptForce prioritization of interventions, *fabZ* and acyl-ACP thioesterase were upregulated and *fadD* was deleted to construct at a strain that produces 1.70 g/L and 0.14 g fatty acid/g glucose (~ 39% maximum theoretical yield) of $C_{14\text{-}16}$ fatty acid in minimal M9 medium (see Figure 3). These results highlight the benefit of using computational strain design and flux analysis tools in the design of recombinant strains of *E. coli* to produce free fatty acids.

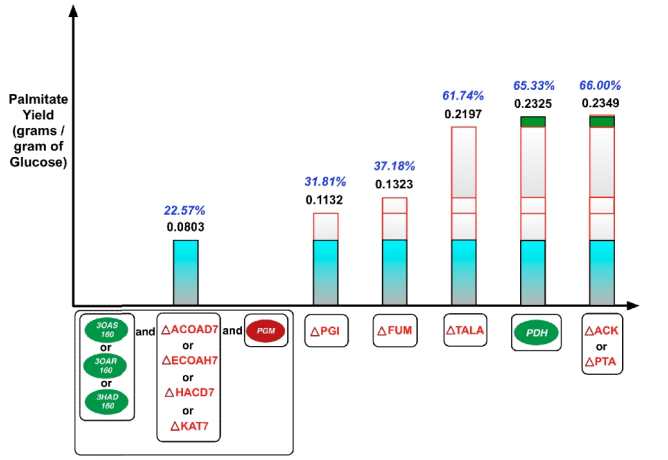

Figure 2. Impact of each genetic intervention predicted by OptForce on the yield of palmitic acid.

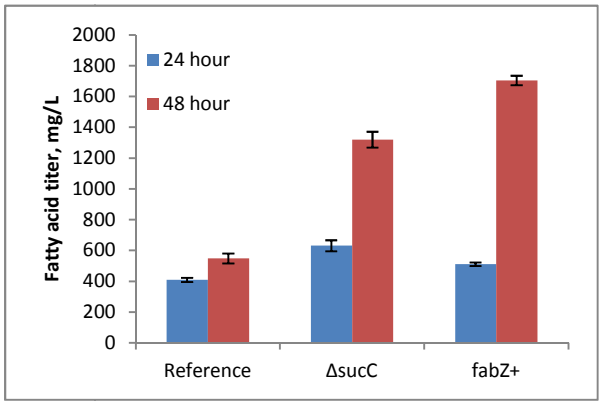

Figure 3. Accumulation of free fatty acids by ML103 pXZ18 (ΔfadD), MLK163 pXZ18 (ΔfadD, ΔsucC) and ML103pXZ18z (ΔfadD, fabZ^{+}) at 24 and 48 hours

Second Generation of Strain Design

In this study, we compare the phenotype and flux distribution of the first generation engineered strain ML103 pXZ18Z (fatty acid degradation knockout, fatty acid elongation and thioesterase overexpression) under the control and fatty acid producing condition, along with transcriptomics and metabolomics analysis. We then demonstrate an iterative metabolic engineering effort that integrates computationally driven predictions and metabolic flux analysis techniques for further improving fatty acid yields. We first construct the new MFA data for the first generation mutant and subsequently redeploy the OptForce procedure to identify additional interventions for improving middle-chain fatty acids production. Figure 4 shows the isotopomer flux data fatty acid producing mutant ML103pXZ18z (ΔfadD, fabZ^{+}). The figure shows that the oxidative TCA cycle activities to be upregulated ~40%, while reactions in the pentose phosphate (PP) pathway were downregulated ~60%. About 50% more carbon fluxes flow through pyruvate kinase, instead of going through phosphoenolpyruvate carboxylase (ppc) pathway. With the decrease in cell growth rate during fatty acid production, the amino acid biosynthesis reactions are generally lower than the control. The increase of fluxes from acetyl-coA to fatty acid synthesis is accompanied with the decrease of flux towards acetate (byproduct formation). The acetate is produced through acetate kinase from pyruvate instead of pyruvate oxidase from pyruvate. In addition, the ED pathway has negligible flux value as well as glyoxylate pathways. The net productions of ATP, NADH and NADPH per glucose consumed increased ~12-20% under fatty acid producing condition.

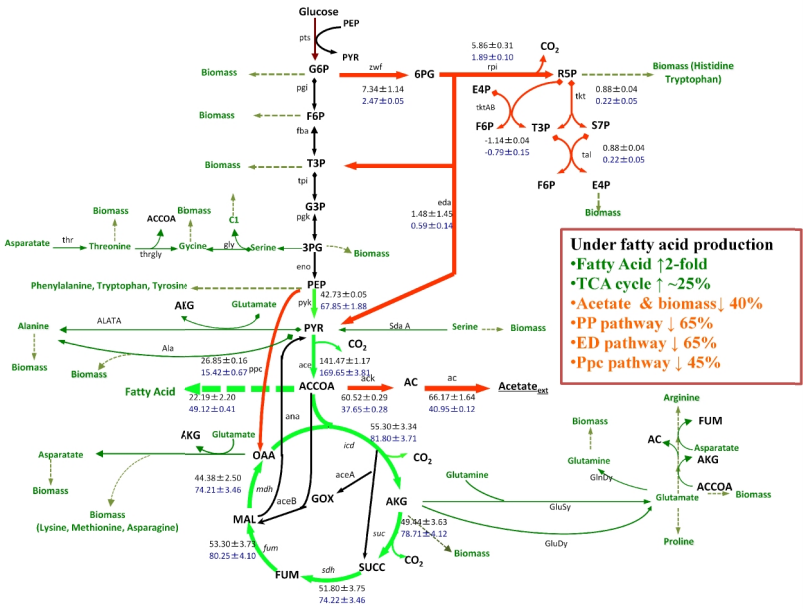

Figure 4 *In vivo* metabolic flux distribution for *E.coli* ML103 pXZ18Z under control condition (black font) and fatty acid producing condition (red font).

In another independent experiment, we investigate the relative change in concentrations of intracellular metabolites (Figure 5) of *E.coli* ML103 pXZ18Z under the control and fatty acid producing condition. The strain was grown in the shake flasks in M9 minimal media with 1.5% glucose. The strain produces up to 2.3g/L fatty acids after 72 hour cultivation. Under fatty acid production, most of the intracellular metabolites in the TCA cycle (i.e. succinate, fumarate and malate) exhibit significant elevation in concentration at 48 and 72 hours compared to the control condition. However, citrate level during fatty acid production is lower than the control. Notably, the increase in TCA cycle metabolite concentration positively correlates with the increase in the TCA cycle fluxes.

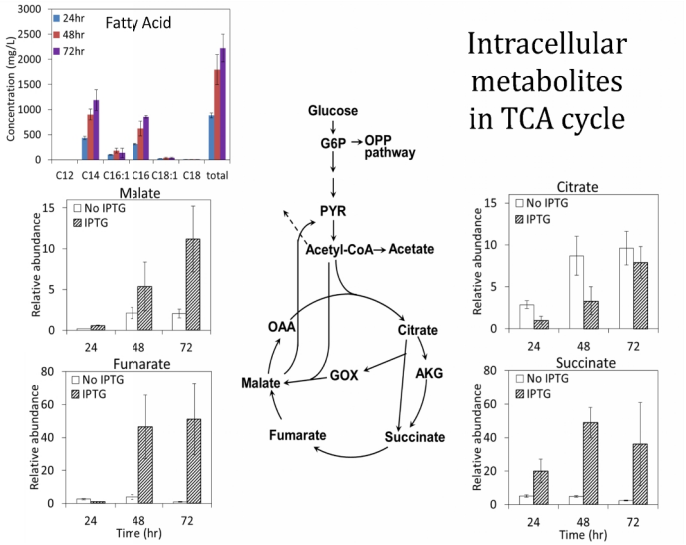

Figure 5 Fatty acid production and relative concentration of intracellular metabolites in the TCA cycle of *E.coli* ML103 pXZ18Z (ΔfadD, fabZ and thioesterase overexpressed) under control condition and fatty acid producing (induced by 1mM IPTG) condition.

As mentioned earlier, the first generation engineered mutant was considered as the new reference strain, and reference phenotype was re-characterized using the MFA data of the mutant. All interventions engineered in the first step (i.e. ΔfadD, fabZ$^+$, and expression of a heterologous thioesterase from *R. communis*) were implemented as suitable constraints in all computational steps. The new set of interventions reflect the new priorities as the new base strain is not wild-type but the first generation mutant. Figure 6 shows the updated predictions of OptForce for the mutant.

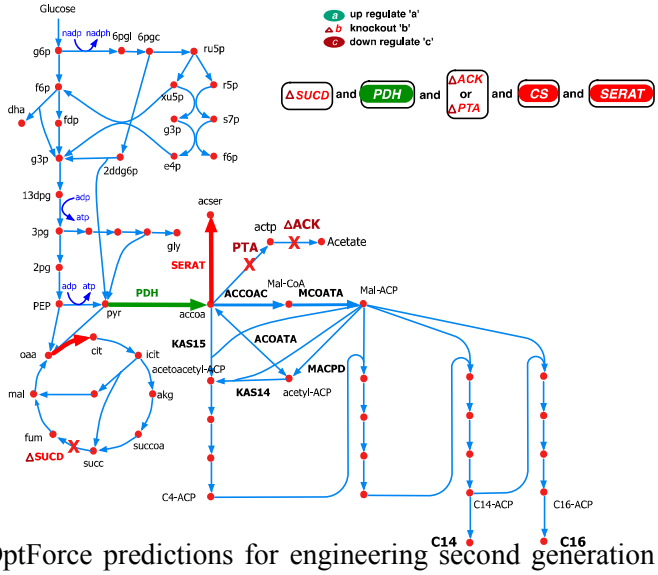

Figure 6. Modified OptForce predictions for engineering second generation mutant for middle-chain fatty acid overproduction.

When this set of results is contrasted with OptForce predictions, we see a change in the prioritization of the interventions according to phenotype changes. As TCA activity increases in the mutant, removal of succinate dehydrogenase (SUCD) reaction is identified as the most important intervention to channel flux towards fatty acids. Similarly, the interventions redirecting metabolic flux towards PP pathway do not appear in the new set of interventions since MFA data shows decreased activity towards it.

The relative increase in fatty acid yield with each intervention is illustrated in Figure 7. Our study reinforces the advantage of integrating computational, experimental and omics tools for the design and engineering of microbial strains to overproduce value-added chemicals or biofuels.

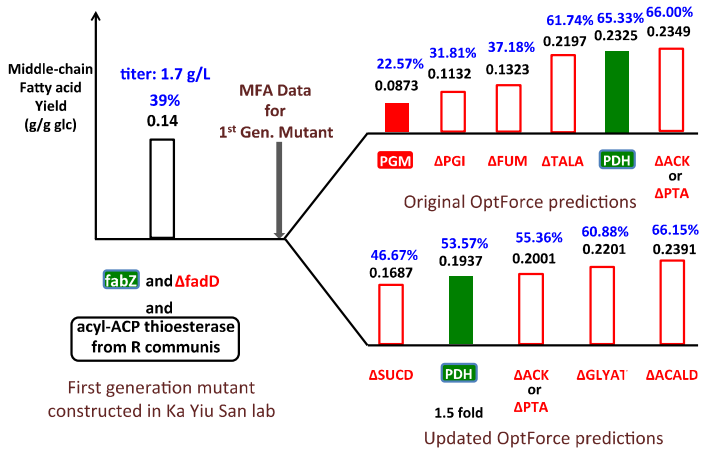

Figure 7. Comparison of the impact of each intervention on fatty acid yield for original and updated OptForce prediction.

Other Relevant Work

Flux analysis methods in yeast and plant systems leverage flux tool development for *E. coli*.

Plans for the Next Five Years

CBiRC generated flux data (ISU) is used in OptForce (Penn State) and FBA models made from BioMart (ISU) to predict necessary flux alterations for optimal productivity of a given chain length. MFA of *E. coli* strains engineered at Rice University to improve fatty acid synthesis will be compared with wild-type and in silico predictions. MFA will be performed for strains engineered for new testbed products. In addition, we will be researching on development of computational protocols for integrating fluxomic, transcriptomic and proteomic data to better understand the cell phenotype and improve the identification of genetic intervention strategies. Finally, we are collaborating with the Bioinformatics project for incorporation of flux data into BioMart for ease of comparison of transcript and other physiological data.

Expected Milestones and Deliverables

Predictions of flux design targets for fatty acid production as the chain length changes from C16 to C6. Integrated metabolic flux platform (simulation and experiment) and biosystems design of *E. coli* strains engineered to improve short chain fatty acid synthesis.

Member Company Benefits

CBiRC member companies, especially the biotechnology start-ups, have expertise in construction of strains but do not have the resources for such detailed flux analysis. The expertise required for experimental and computational flux analysis is not trivial and in short supply. Our team has extensive experience in experimental metabolic flux analysis, constructing genome-scale metabolic models and computational metabolic flux analysis and optimization procedures. The integrated flux platform is being demonstrated in CBiRC testbeds, and will create a powerful tool for strain design and optimization.

Commercialization / Technology Transfer

We are actively engaging with Center member companies, including start-ups, in discussion of the integrated flux platform with their research needs.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.4B -** Flux Analysis in *S. cerevisiae*

Thrust: Research Thrust 2 - Microbial Metabolic Engineering

Pursuant to guidelines for preparing ERC annual reports, three- to five-page Project Summaries are required for all core and sponsored projects (those with direct support from the Center). A Project Summary should also be provided for each supplementary and special-purpose award received by the ERC. Project Summaries do not have to be included for proprietary projects where such a summary would compromise the sponsor's interests. In general, Project Summaries are NOT required for *associated* projects; rather, abstracts and other information for these projects will be collected separately. However, for Gen-3 ERCs, foreign partner associated projects may include a Project summary rather than only an abstract if the project is of particular importance to achieving the vision of the center.

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Jacqueline V. Shanks	**02/28/2013**	**03/01/2012 to 02/28/2013**

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Jong Moon Yoon, Department of Chemical and Biological Engineering, ISU

Statement of Project Goals

The goal of the project is to construct metabolic flux maps for *S. cerevisiae*, for both the wild-type and engineered strains and under various operating conditions. The flux maps from this project will be used to guide further genetic manipulations for strain improvement.

Project's Role in Center's Strategic Plan

Metabolic flux maps are an integral part of the metabolic engineering design cycle to construct strains that produce carboxylic acids and pyrones, precursor chemicals that require catalysis via Thrust 3 for the synthesis of α-olefins, dienes, and other compounds. Metabolic fluxes are an important physiological characteristic, providing a global perspective of the integrated functioning between levels of transcripts, proteins, and metabolites to cellular phenotype. Metabolic flux analysis identifies potential bottlenecks in the reaction network that limit production of the target compound. These bottlenecks are then genetically engineered out in the next metabolic engineering cycle.

Fundamental Barriers and Methodologies

A key barrier in the overall goals of Thrust 2 will be to shorten the metabolic engineering cycle. Since

flux plays an integral role in the metabolic engineering cycle, this means a quick turnaround time for flux analysis results to the strain construction and bioinformatics projects. Fundamental barriers for metabolic flux analysis lie in (1) the validation of the flux map, (2) in deciding the right metabolic flux analysis mapping tool for the application, and in (3) the correct basis of the metabolic flux results to integrate into the bioinformatics framework for comparison to other data sets. For validation of the flux map, the network topology and nomenclature is coordinated with the Bioinformatics project that mines *S. cerevisiae* data. In deciding the tradeoff in the time intensive but information rich comprehensive flux analysis versus a more high-throughput "fluxomics" method (which either only uses partial labeling information to obtain a flux map or correlates labeling information via a bioinformatics approach) an assessment of conventional MFA and fluxomics MFA, was benchmarked with comprehensive MFA, so that a design strategy can be assessed so that more strains can be characterized at the level needed.

Achievements

Octanoic Acid Toxicity MFA of BY4741

Comprehensive and reproducible flux maps of *S.cerevisiae* BY4741 under control conditions and toxicity challenge (0.4mM octanoic acid) in SD minimal media were obtained. Aerobic batch cultures were conducted in a Multifor system with working volume 400 ml, at 30°C, with 600rpm agitation and pH control to 5.0. The dissolved oxygen (DO) level was kept above 50% saturation during the entire fermentation to ensure aerobic conditions.

Phenotype differences were observed when the *S. cerevisiae* cells were under 0.4mM octanoic acid during aerobic batch fermentation as shown in Figure 2. The cells exhibited respiratory-fermentative metabolism with secretion of ethanol, acetate and glycerol. Under octanoic acid exposure, cells grew slower with ~25% lower specific growth rate and slightly higher specific glucose uptake rate. Ethanol and acetate yield increased by 10% and 2-fold respectively, coupled with lower biomass yield of 41% under C8 fatty acid inhibition. However, glycerol yield decreased ~7-fold under octanoic acid inhibition. Notably, glycerol plays improtant roles in yeast physiological processes such as combating osmotic stress, managing cytosolic phosphate levels and maintaining the NAD+/NADH redox.

Since the yeast strain is auxotrophic for histidine, leucine and methionine, we quantified the residue concentrations of the amino acids to obtain amino acid uptake rate (Figure 3). Leucine was uptaken the most under the control condition (~0.7mM/g.hr). Compared to the glucose uptake rate 0f ~17mM/g.hr, leucine uptake is just ~4% of the total carbon substrate, thus negligible to affect the flux distributions. Octanoic acid was also quantified using GC-MS, but we found no significant evidence of octanoic acid oxidation that is being degraded and uptaken by the cells.

When cells were exposed to 0.4mM octanoic acid stress, the oxidative TCA cycle activities were observed to be upregulated ~12 fold while reactions in the pentose phosphate (PP) pathway was rigid. The glycerol secretion decreased by ~85%, leading to ~10% more fluxes flowing through lower glycolysis pathway. At the pyruvate node, pyruvate dehydrogenase converting pyruvate to acetyl-coA increased 11-fold, while pyruvate carboxylase that converts pyruvate to oxaloacetate was inhibited by ~40% under octanoic acid stress. With the increase in ethanol and acetate yield, carbon fluxes through pyruvate decarboxylase slight increased ~6%. In addition, along the decrease of cell growth rate during fatty acid stress, the amino acid biosynthesis reactions were generally lower than the control. The net productions of ATP per glucose consumed were upregulated 20% under fatty acid stress (Figure 3). The net production of NADH and NADPH are 5-fold higher and 13% lower respectively, leading to ~2.8 fold increase in the overall reducing power NAD(P)H under octanoic acid inhibition suggesting possible important roles of the cofactors in defending the cell growth from octanoic acid toxicity.

Glycerol plays important roles in physiological processes such as combating osmotic stress, managing

cytosolic phosphate levels and maintaining the NAD+/NADH redox balance. Since extracellular octanoic acid might disrupt the function of glycerol production, knock-out strains of key genes in glycerol formation were challenged with 0.3 mM of C8 in the SD minimal media. However, the gdp and gpp gene knock-out in *S. cerevisiae* exhibited the same reduction in growth as observed in the wild-type strain. This led to proposition that octanoic acid might not be due to increased osmotic stress but acidification of intracellular cytosol resulting in membrane stress.

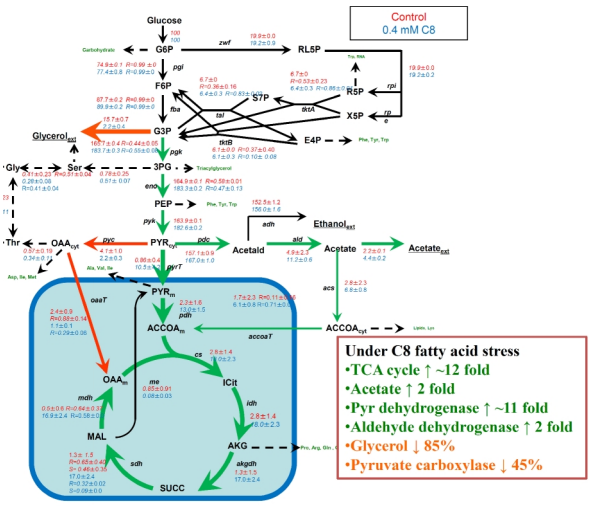


Figure 4 *In vivo* metabolic flux distribution for *S.cerevisiae* BY4741 under control condition (red font) and 0.4mM octanoic acid stress (blue font). Estimated fluxes are normalized to 100 mmol/g DCW.hr based on the average specific glucose uptake rates. The flux values shown are average from two replicates ± standard deviation.

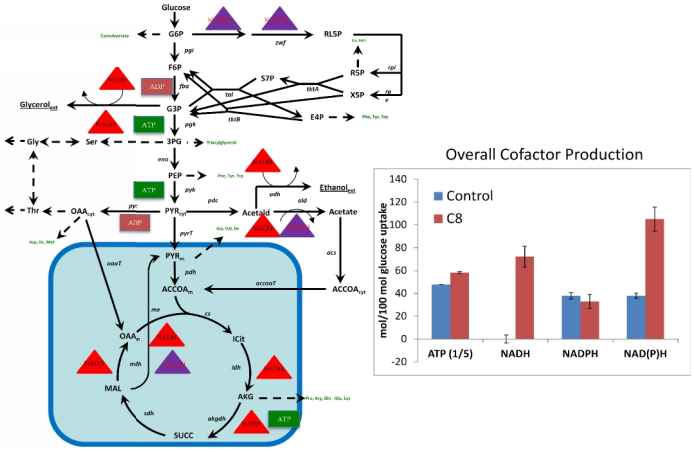


Figure 5 Cofactor (NADH, NADPH) and energy (ATP) production in central carbon metabolism. The red triangle represents NADH, the purple triangle represents NADPH and the green rectangular represents ATP. The total production in central carbon metabolism for control and fatty acid producing conditions is show in the graph. Malic enzyme activity and aldehyde dehydrogenase involve both NADH and NADPH, the overall reducing power production can be captured by NAD(P)H.

Reconcile Flux Results with Transcriptomic Analysis

We performed transcriptome analysis of *S. cerevisiae* BY4741 during mid-log growth in SDC media with and without exposure to 0.3mM C8, which is sufficient to decrease the specific growth rate by 25%. This analysis identified 937 genes with significantly ($p<0.01$) perturbed expression in the +C8 condition relative to the control; 136 of these genes have expression that is perturbed more than 2-fold.

Most of the genes in the central carbon metabolism do not change significant except TKT1 in the oxidative PP pathway and MAE1 in the malic enzyme activity. The TKT1 and MAE1 genes were downregulated 13.8 times and 2.1 times respectively under octanoic acid inhibition, but the corresponding reaction fluxes of transketolase and malate dehydrogenase did not perturb. Interesting, the genes related to plasma membrane ATP-binding cassette transporter such as PDR3, PDR12, PDR15 and PDR16 were upregulated significantly (2-5 times higher than the control). This implied octanoic acid stress might acidify the intracellular pH of the cytosol and mitochondrion and then disrupt the integrity of cell membrane, thus activating the plasma membrane transporter to repel the toxin out of the cells as shown in Figure 3. The hypotheses was supported the increase in TCA cycle activities to generate more ATPs for H^+ ATPase activity to efflux the toxic anion through plasma membrane ATP binding cassette transporter.

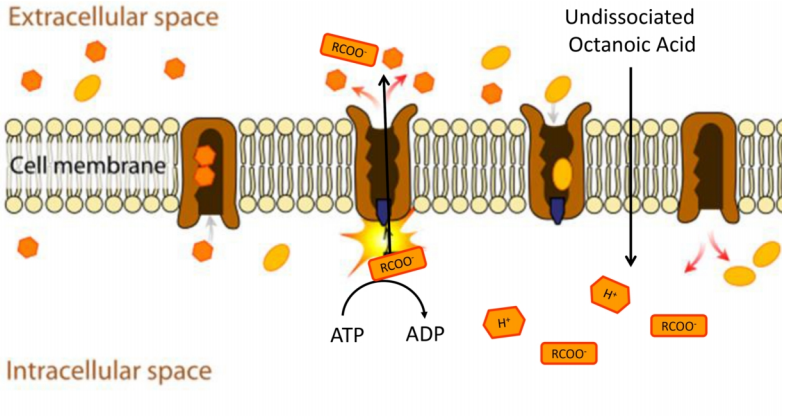


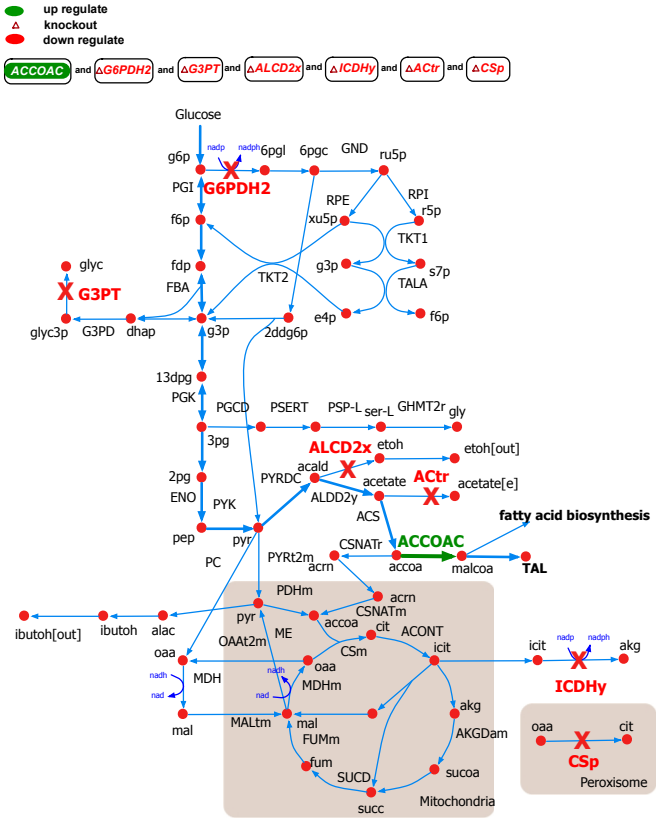
Figure 3 Mechanism of acidification of intracellular space by octanoic acid and activation of ATPase to repel the toxin.

Integrating OptForce and Flux Data for Pyrone Production

We used the *i*AZ900 metabolic model of *S. cerevisiae* for the OptForce calculations. Preliminary calculations revealed that *i*AZ900 contained a number of thermodynamically infeasible cycles, which were subsequently identified and manually removed. Metabolic flux data was obtained from Gombert *et al* (2001) to define the phenotype of the wild-type strain of *S. cerevisiae*. The OptForce procedure was subsequently implemented to identify a minimum set of genetic interventions for maximizing the production of TAL. Figure 4 shows the intervention strategy identified by OptForce for TAL overproduction. Besides augmenting the availability of malonyl coA, which is the direct precursor for TAL production, OptForce suggests removal of competitive pathways draining away metabolic flux. We also see a systemic reduction in NADPH production to prevent the activity of competitive fatty acid synthesis.

Figure 4: OptForce suggested interventions for overproduction of TAL in *S. cerevisiae.*

The simulations also revealed that the balance of NADH cofactor in *S. cerevisiae* has significant impact both on cell growth as well as TAL production. Removal of ethanol production pathway, as



predicted by OptForce, eliminates the main sink of NADH in *S. cerevisiae*. The inability of the metabolic network to find a suitable alternative sink for NADH in the cytosol, coupled with a lack of a suitable transport mechanism for cytosolic NADH into mitochondria, sets significant barriers in reconfiguring the metabolic network for TAL overproduction. The OptForce simulations allude to the need to engineer an alternative sink for NADH to improve production of TAL.

Other Relevant Work

Flux analysis methods in plant and *E. coli* systems leverage flux tool development for microbial systems.

Plans for the Next Five Years

MFA of *S. cerevisiae* strains engineered at University of Irvine to improve short-chain fatty acid synthesis will be compared with wild-type. Flux analyses of strains exposed to toxic levels of short-chain fatty acids are being performed in parallel with transcriptomic experiments and bioinformatic analysis in order to enhance tolerance to C6 and C8 fatty acids. CBiRC generated flux data (ISU) is used in OptForce (Penn State) to predict necessary flux alterations for optimal productivity of pyrones in engineered *S. cerevisiae* strains (Irvine). OptForce will also be employed to study the improvement in TAL production on expression of heterologous pathways in the host for improved conversion of pyruvate towards acetyl-coA as well as improving the NADH cofactor balance in cytosol of *S. cerevisiae*. MFA will be performed for strains engineered for new testbed products.

Expected Milestones and Deliverables

With collaboration with the omics project, we will perform transcriptomic experiments in conjunction with

the ^{13}C labeling experiment to help test hypotheses of mechanisms of fatty acid toxicity.

Integrated metabolic flux platform (simulation and experiment) and biosystems design of *S. cerevisiae* strains engineered to improve short chain fatty acid or pyrone synthesis.

Member Company Benefits

CBiRC member companies, especially the biotechnology start-ups, have expertise in construction of strains but do not have the resources for such detailed flux analysis. The expertise required for experimental and computational flux analysis is not trivial and in short supply. Our team has extensive experience in experimental metabolic flux analysis, constructing genome-scale metabolic models and computational metabolic flux analysis and optimization procedures. The integrated flux platform is being demonstrated in CBiRC testbeds, and will create a powerful tool for strain design and optimization.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.5A** - Bioinformatics in *E. Coli*

Thrust: Research Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Julie A. Dickerson	**02/27/2013**	**03/01/2012 to 02/28/2013**

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Graduate Students: Erin Boggess (ISU), Al Yao Fu (ISU), Liam Royce (ISU), Jesse Walsh (ISU)

Undergraduate Students: Kara Moeller (ISU)

Statement of Project Goals

Develop models to integrate in-house omics data with existing databases to provide a system-wide view of the production strains. Develop tools based on a systems-wide approach to provide insights and suggestions for further strain improvement.

Project's Role in Center's Strategic Plan

The bioinformatics tools developed in this project will provide a new model for improving strains and achieving optimized product production. Genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the products from Thrust 2 will serve as precursors for the synthesis of α-olefins and dienes by Thrust 3, the Chemical Catalysis group.

Fundamental Barriers and Methodologies

Meaningful data integration across heterogeneous data sources is difficult to achieve as the importance and reliability of data sources is unknown at this time. Additional problems include incomplete and uncertain data on the structure of the metabolic networks under different conditions.

Achievements

In order to promote greater sharing of knowledge and improve interdisciplinary training for the graduate students, the *E. coli* omics experiments and bioinformatics teams have been meeting weekly since the beginning of the project. These discussions have reviewed key pathways in E. coli central metabolism, bioinformatics tools for omics data, and of metabolic flux analysis. The bioinformatics team has been using existing *E. coli* datasets to explore different bioinformatics tools for analysis of gene regulatory networks.

1 Mutation Analysis and Strain Design

In metabolic engineering, a bottleneck occurs when mapping mutations to phenotype or vice versa. Strain design is often restricted to gene overexpression and gene knockouts with the goal increasing or turning off target reactions. This binary approach may not produce optimal strains and can suffer from limitations of required plasmids. Enzyme engineering and modification of regulatory entities such as promoters and transcription factor binding sites offer opportunities to fine-tune cellular activity. Strain development via metabolic evolution also struggles to relate mutations in evolved strains to improved fitness. Mutation annotation is a difficult manual process that significantly increases to a secondary round of adaptive evolution. The massive amount of sequence variation data generated in evolution experiments necessitates computational tools that assess mutation implications. Regulatory entities must also be included in the analysis for accurate interpretation.

With the goal of improving upon both processes of strain design, we propose a framework to systematically analyze mutations and provide interpretations for both direct impact and downstream effects. Genes, products, regulatory elements, interactions, and relationships of these entities are included in the framework. This data is obtained from public databases such as EcoCyc, RegulonDB, and PDB. The proposed analysis framework benefits the research community by broadening the study of mutations and mechanisms of adaptation. Additionally, automating portions of comparative genomic analysis reduces the lifecycle of adaptive evolution studies. The rational design process also benefits from the framework by the ability to identify regulatory entities associated with a specific protein. For example, if an enzyme is a target for manipulation, elements involved in transcription and translation of the required proteins can be targets for controlling enzyme abundance.

2 Global Regulatory Network Model Integrating Multiple Interaction Networks in *E. coli*

The research on regulatory networks of *E. coli* is currently focusing on integrating multiple types of interaction networks, including TF-gene interactions, sRNA-gene interactions, protein-protein interactions, protein-ligand interactions, and other metabolic reactions, to construct a global regulatory network model and reflect the regulatory signal transduction within the whole cell. During the integration, instead of using traditional chemical reaction for to represent metabolic reactions, metabolic reactions are transformed into interactions to show regulatory relationships between compounds. Analysis such as network properties and network motif can improve understanding of the regulatory of *E. coli* as a whole system. Combining with CBiRC -specific data, this global regulatory network model helps understand experimental results and phenotypes, as well as predicting the regulatory response of genetic engineering under certain environment conditions.

3 Transcriptome Data Analysis

As part of Thrust 2.4A Omics Analysis, the Jarboe lab performed two experiments and associated RNA extractions for high throughput RNA sequencing. The first examined the effects of the addition of 35 mM octanoic acid on the *E. coli* K-12 MG1655 strain. The second experiment examined the production strain, ML103 + pXZ18Z (Δ*fadD*, *fabZ*, and thioesterase), with and without the inducer IPTG.

3.1 C8 fatty acid stress experiment

For the C8 stress experiment, three control and three treatment RNA samples were sequenced using paired-end reads of 51 bp in length were generated using the Illumina GAII RNA-Seq platform. Analysis of the output was performed using the Tuxedo protocol. First, short reads from each sample were aligned to the wild-type transcriptome, *Escherichia coli* K-12 MG1655, using its published genome and a custom gene model annotation file generated from the latest version of EcoCyc. Next, alignment information is used to quantify transcript abundance for all annotated genes. Finally, CuffDiff calculates fold change of transcripts across multiple conditions. For analysis, the three control samples and the three 35 mM C8 treatment samples were pooled to perform the comparison between the two conditions. The comparison between 0 and 35 mM C8 identified 207 genes with an FDR corrected p-value < 0.05 and $|\log_2(\text{fold change})| \geq 2.0$ (117 upregulated with and 90 downregulated). Examining overrepresentation of Gene Ontology terms revealed that many of these genes corresponded to response to oxidative stress, acid response, response to pH, taxis, and cell motility. Network Component Analysis (NCA) was in concordance with these findings as transcription factors such as FlhDC (principal regulator of bacterial flagellum biogenesis) and GadX (principal regulator of the acid resistance system) had the largest predicted changes in activity.

Transcription Factor Activities (TFAs)

Using the TFA estimation methods as described in previous reports, TFA of the 35 mM C8 data was estimated from the RNA-Seq experiment results.

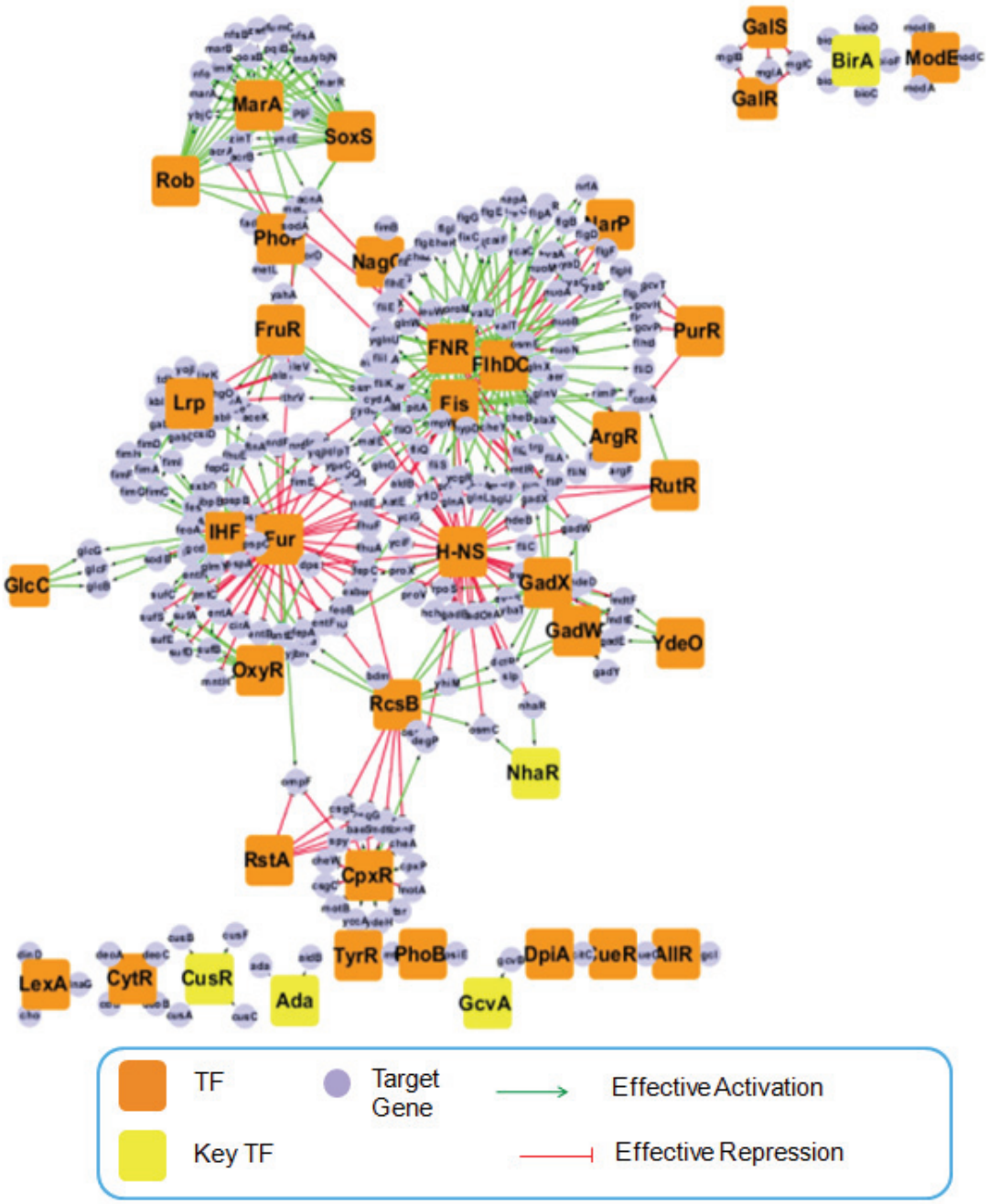
TF	Fold Change	p-value	# perturbed target genes	Sensing Signal
GadX	2.0093	0.0001	14	Low PH
CpxR	1.6285	0.0001	18	Alkaline pH, altered membrane lipid composition, interaction with hydrophobic surfaces, and high osmolarity.
FlhDC	-4.6617	0.0004	40	High osmolarity, catabolic repression, quorum, synthesis of type 1 fimbriae, the histone-like nucleoid protein, heat shock, etc.
FNR	-1.7540	0.0025	36	Oxygen
BirA	-2.3864	0.0026	5	Biotin
CusR	-1.2849	0.0038	4	Copper, silver and histidine
Fur	-1.0963	0.0170	39	Concentration of intracellular iron, and metal ions
GadW	2.6845	0.0258	6	Low PH
NarP	-1.0842	0.0395	5	Nitrate and nitrite

Significantly changed TFAs under 35 mM C8 fatty acid stress. Comparing to the TFA

behavior of *E. coli* under 10 mM C8 fatty acid stress we had in last year's report, the gene regulatory system under 35 mM C8 shows more responses to low PH (1 low PH sensing TF has significant TFA change under 10 mM C8, at least 2 low PH sensing TFs have TFA change under 35 mM C8). The significant TFA change of CpxR indicates the altered membrane lipid composition. The significant responses of many ion and small molecular sensing TFs, such as FNR, BirA, CusR, Fur and NarP, suggests that there might be some difficulties of the cell to manage the concentration small moleculars in the cells, and possibly due to the membrane damages or membrane dysfunction.

Analysis of regulatory network behavior

To learn the how the GRN effectively regulate gene expressions according to experimental condition changes, the Effective Regulatory Network (ERN) model focuses on the effective part of GRN and reflects the dynamic change of GRN while experimental condition changes. The ERN is constructed by regulatory links between TFA significantly changed TFs and differentially expressed genes. Key TFs are identified based on ERNs. Key TFs at specific experimental condition changes are TFs which significantly change network properties, efficiently rewire GRN, and successfully regulate target genes.



The figure above shows the Effective Regulatory Network and Key TFs of *E. coli* under C8 fatty acid stress. According to the figure, there are three major regulatory systems are actively responding to the C8 fatty acid stress: general stress response (FlhDC, Fis, and H-NS), acid resistance (GadX, GadW, and RcsB), and membrane function related (CpxR, CusR, NhaR,and BirA).

3.2 IPTG experiment

For the IPTG experiment, three control and three treatment RNA samples were sequenced using the Illumina HiSeq platform. Paired-end reads of 100 bp in length were generated.

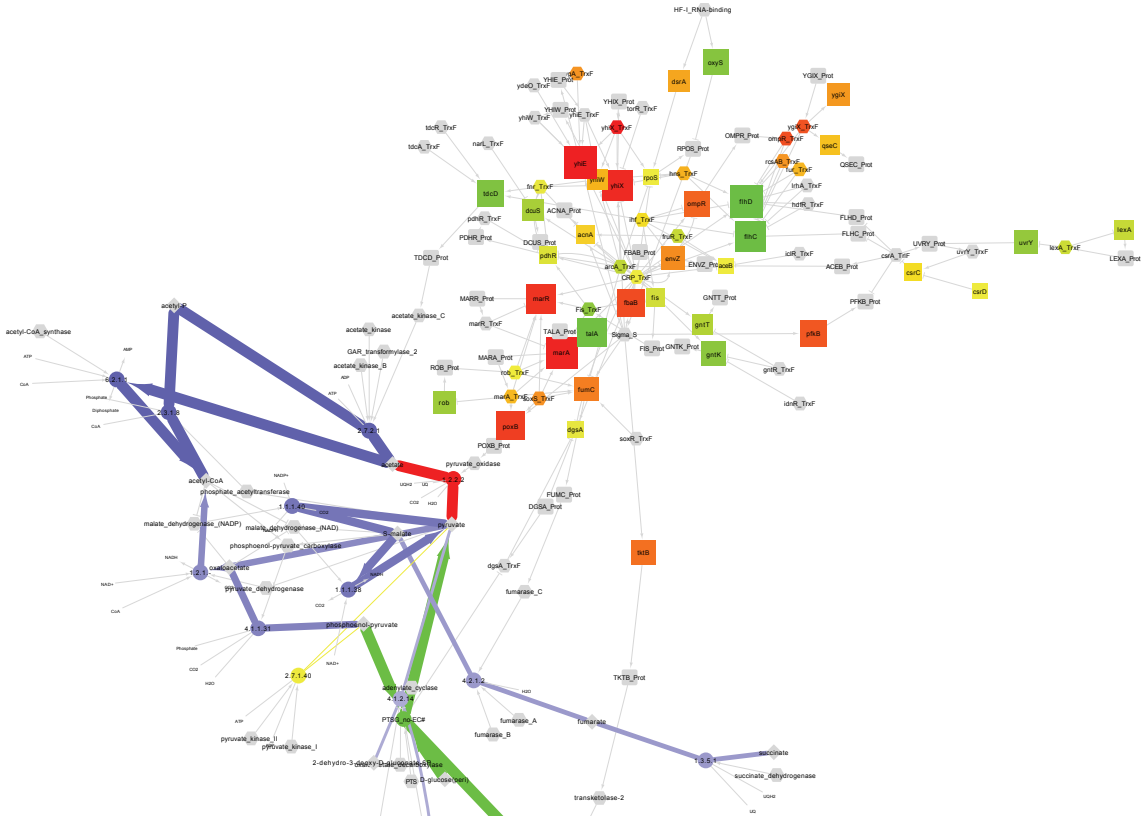
Control and treatment data were pooled and same Tuxedo protocol was used for analysis. The comparison between 0 and 1 mM IPTG identified 149 genes with an FDR corrected p-value < 0.05 and $|\log_2(\text{fold change})| \geq 2.0$. Examination Gene Ontology annotations for these genes revealed themes such as stress and acid response, membrane proteins, transporters, biofilm production and colanic acid production. Additionally, Network Component Analysis (NCA) was performed for this subset of 149 genes and two proposed transcription factor-gene connectivity matrices generated by Yao Fu of Dr. Dickerson's lab. Each analysis resulted in nine transcription factors with significant predicted activity changes: ArgR, CRP, FlhDC, FNR, Fur, H-NS, LexA, NtrC, and RcsAB. Based on these results, additional acidity and membrane experiments are being performed by the Jarboe lab in order to develop methods to improve tolerance and stabilize membrane fluidity.

4 RNA-seq and Flux Data Integration

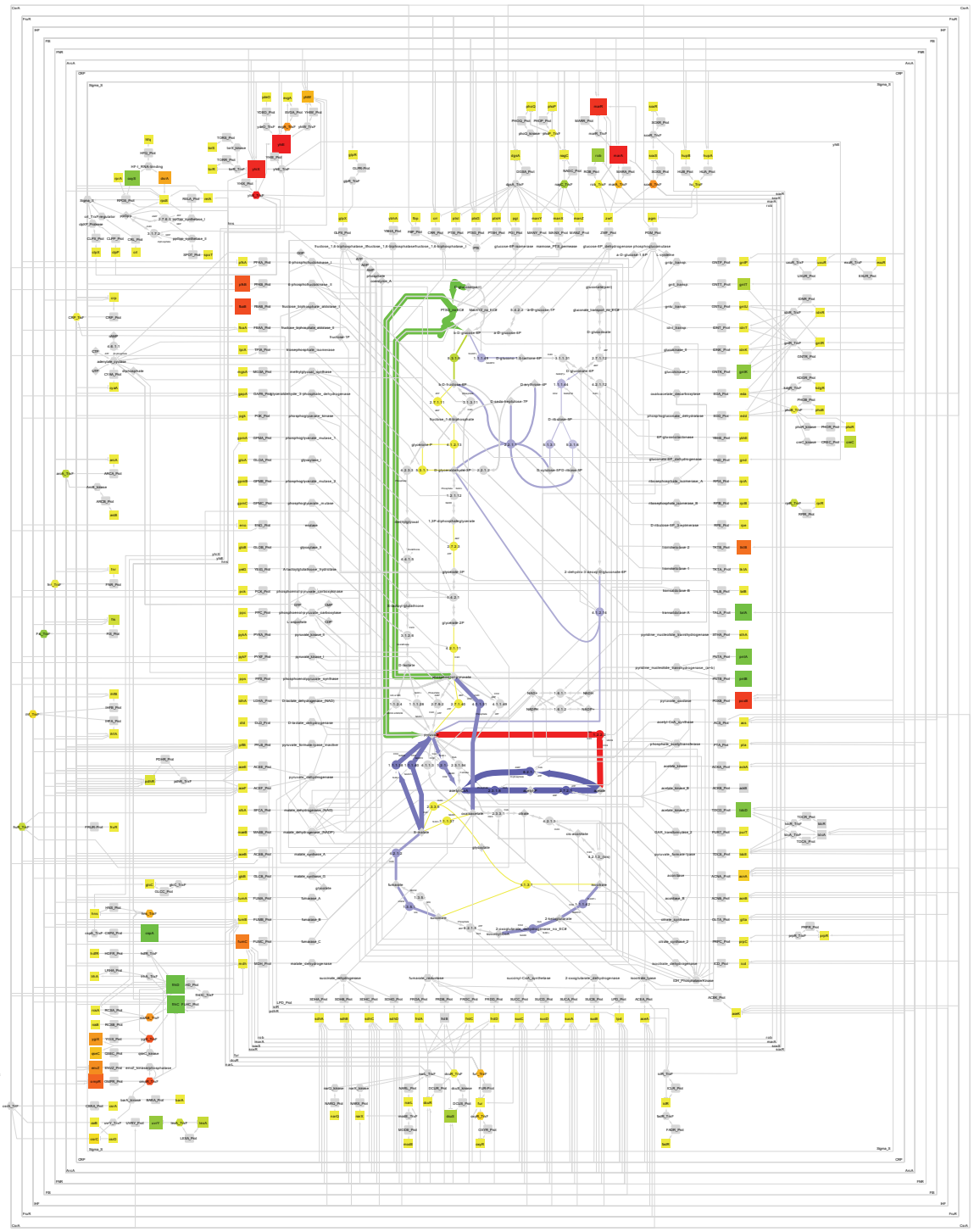
In addition to mRNA quantification, ^{13}C metabolic flux analysis was performed for the 0 vs. 35 mM octanoic acid experiment. Flux and transcript data are traditionally analyzed independently, however it is desirable to combine the results to obtain a better understanding of cellular activity in what is called "multi-omics" analysis. Because flux and transcript quantifications are wholly different metrics, ratios between control and treatment conditions were used for an integrated analysis. These ratios were mapped onto the central carbon metabolism of *E. coli* along with predicted transcription factor activity changes predicted from the network component analysis algorithm.

A visualization of all collected data was created using the MODAM plugin in Cytoscape. A total of 363 data points were successfully mapped to the central carbon metabolism (31 reactions, 302 transcripts, and 30 predicted transcription factor activities). Visual integration of multiple types of information allows for a global analysis that provides a general sense of data concordance. Discrepancies may indicate inaccurate/incomplete metabolic models or hidden influences such as post-translational modification, post-transcriptional regulation, cofactors, and environmental effects.

The network generated from octanoic acid stress experiment data identified key nodes such as pyruvate dehydrogenase and the *poxB* gene that were identified as having significant activity in previous work examining short chain fatty acid stress. The integrated network also revealed nodes with high connectivity to entities that exhibited significant change for which there was no measured (or predicted) data. The unobserved data points offer targets for follow up experiments and may help identify new regulatory activities. Additionally, deviation between transcript and flux can provide a basis for model refactoring and model constraints. The ability to improve flux models is critical for engineered strains with altered or novel pathways.



Subnetwork generated by MODAM illustrating increase in poxB/pyruvate dehydrogenase and the fold changes of related entities (0 vs 35 mM C8 experiment)



Central carbon metabolism of *E. coli* (0 vs 35 mM C8 experiment) with flux, transcript, and predicted TFA fold changes.

5 Strain Specific Database

Creation of strain specific databases requires the ability to make both small and large scale changes to a BioCyc database. One example of a large scale change is the inclusion of new

predicted regulatory links. Previously, these large-scale changes were made with a java-based script that did not allow for much flexibility in data type or target database. Improvements to the batch loading tool now allow any spreadsheet formatted file to be mapped to locations in a biocyc database with minimal effort.

5.1 Improving model generation from EcoCyc

As reported previously, genome-scale models of metabolism are generated from EcoCyc by extracting reaction information from EcoCyc and writing to an SBML file. One complication in extracting reaction information from EcoCyc stems from reaction information which defines reactions using non-specific metabolites. These generic reactions must first be converted to instantiated reactions by converting non-specific metabolites to specific metabolites. Performing FBA analysis has previously been infeasible due to the model lacking a media or biomass definition. A definition for minimal media and E. *coli* biomass was adapted from the iAF1260 model [Feist, 2007] by manually mapping the chemical definitions for media and biomass to chemicals in EcoCyc.

Attempts to run FBA on the genome-scale model have failed using these definitions as some of the biomass precursor components were not reachable. Current work will continue to find and unblock biomass precursors in generated genome-scale metabolic model in an effort to show model can predict growth. No generic method is available to identify blockages, and existing tools are inadequate, as they are only able to determine if a single reaction at a time is reachable. As such, manual effort is required to trace expected routes to a particular unreachable metabolite and determine the cause of the block. When all biomass precursors have been unblocked, a comparison to existing growth/no-growth data and gene essentiality data will be performed to assess the predictive quality of the model.

Other Relevant Work

Relevant similar work is also being conducted within CBiRC using yeast as the model microbial system. Many studies have been done to learn gene regulatory networks from microarray data and we are comparing our results for this data. Our work will integrate networks from different sources and combine them with pathway data information to get a more complete picture of interactions.

Plans for the Next Year

The Dickerson lab will continue to work with our collaborating labs to develop new tools for omics data analysis focused around the structure of the metabolic network of *E. coli*. The effective regulatory networks for key conditions will be described and used in metaolic networks.

Expected Milestones and Deliverables

- Integrate flux data and transcriptome data into a more complete *E. coli* metabolic model.
- Improve methods for the reverse engineering in RNAseq datasets for determining the effects of directed evolution.

Member Company Benefits

The visualization tools and the methods for analyzing metabolic networks would be useful for scientists at these companies to quickly assess the results of large-scale omics investigations.

Commercialization / Technology Transfer

Opportunities for technology transfer and commercialization are expected from the work.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.5B -** Bioinformatics in *S. cerevisiae*

Thrust: Research Thrust 2 – Microbial Metabolic Engineering

Prepared By: **Eve Wurtele**	Date *(in U.S. date format)*: **2/17/2013**	Reporting Period: **03/01/2012 to 02/29/2013**

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Statement of Project Goals

Identify new genes that regulate polyketide metabolism in yeast. Develop models to integrate in-house 'omics data with existing databases to provide a system-wide view of the production strains. Develop tools based on a systems-wide approach to provide insights and suggestions for further strain improvement, and to systematically optimize yeast performance for diversion of carbon to synthesis of carboxylic acids and pyrones.

Project's Role in Center's Strategic Plan

The bioinformatics tools developed in this project, and the genes identified, and models developed, provides a new approach for improving strains and achieving optimized product production of polyketides.

Fundamental Barriers and Methodologies

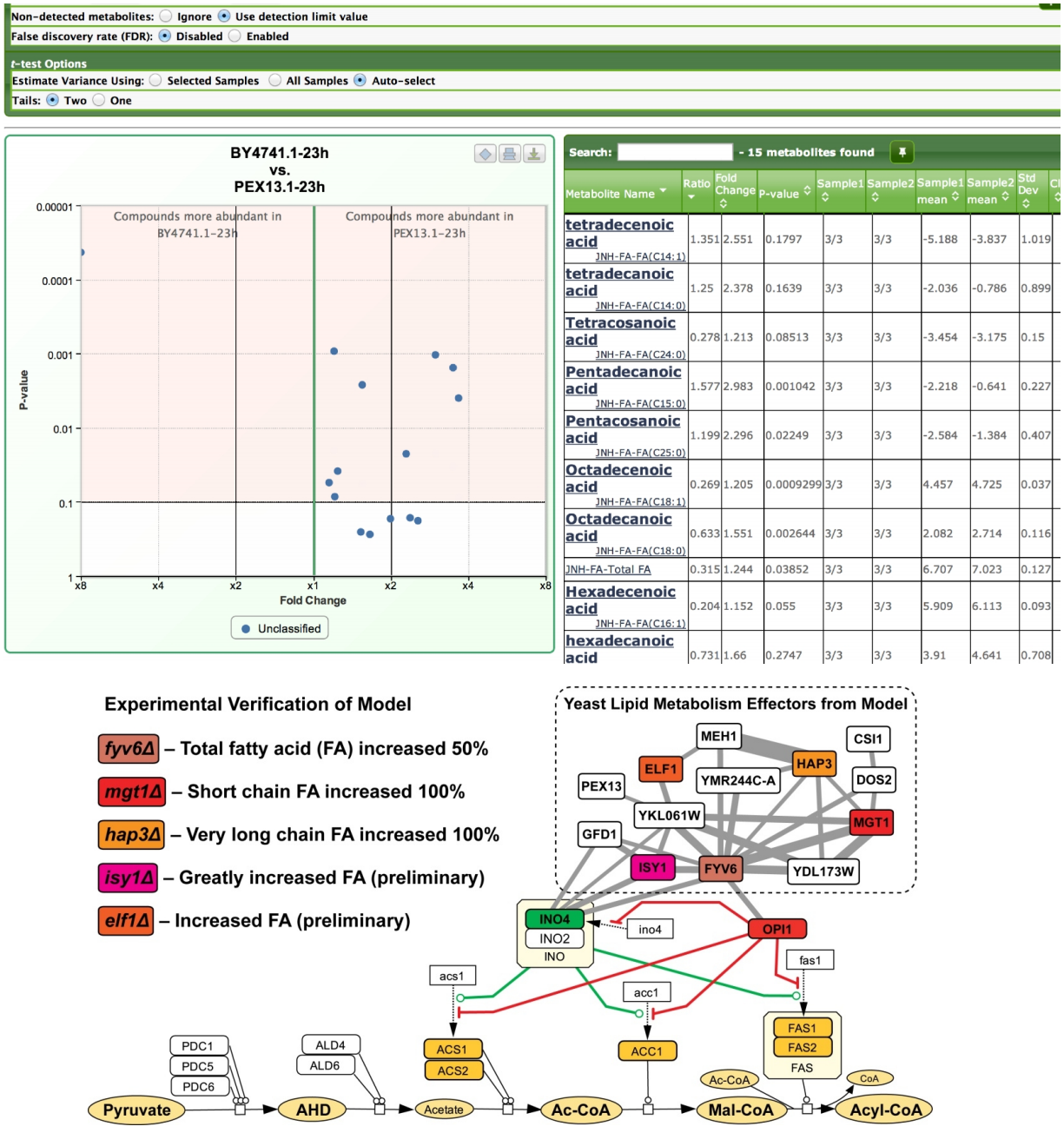
Meaningful data integration across heterogeneous data sources is confounded because the

reliability of data sources and types is difficult to evaluate. Eukaryotic organisms such as yeast have multiple layers of regulatory and metabolic complexity, which prove a challenge for analysis. Surprisingly, much pathway information and gene functional annotation for yeast is still unknown.

Achievement

1. We have computationally identified new genes that we predicted might participate in the fatty acid metabolic network. We have experimentally verified that majority of these genes indeed impact alter fatty acid composition and/or profile in yeast. (specifics are included in achievements 3-9)
2. We have established yeast in the PMR database and analysis tool (PMR was generated via participation in T1 in this funding period), and used PMR to enable interactive data analysis by biochemists. Current data on yeast is available to CBiRC researchers, and will be made publically available upon publication/IP.
3. We have focused on the generation of data on fatty acids and transcripts statistically analyzed in combination with known fatty acid biosynthesis genes to identify genes that are likely to affect yeast lipid metabolism. Additional genes have been added to the model.
4. Based on experimental results from the 15 yeast genes that were implicated in our 2011 yeast model as impacting fatty acid/polyketide profile and content, we extended our research in the yeast knockout lines. Yeast from the various knockout lines were grown in a time course study, and samples were collected at intervals. Samples were analyzed by a combined growth analysis, GC-MS analysis of fatty acid from these mutant strains. Samples were retained for RNAseq analysis on selected strains.
5. Additional yeast strains were made that overexpress the 15 genes in our model. These also display distinctive alterations in fa content and profile.
6. FYV is assigned as a protein of unknown function, and from it's sequence has been proposed to regulate double-strand break repair. The FYV6 mutant induces a 50% increase in total fatty acid.
7. MGT1 is reported to be a DNA repair methyltransferase involved in protection against DNA alkylation damage. The MGT1 mutant induces a 100% increase in those fatty acids of 14-carbons and less.
8. HAP3 is a considered transcriptional activator and global regulator of respiratory gene expression. The HAP3 mutant induces a 100% increase in fatty acid of 20-carbons and greater.
9. PEX is a putative membrane protein and knockouts

Figures:
Above. An example of the PEX KO mutant of *S. cerevisiae*, and altered accumulation of fatty acids. The data is visualized in the PMR database and analysis software.
Below. The overall model, overlaid with the experimental verification to date, is shown below ("Model verification" figure)



Other Relevant Work

Plans for the Next Year

We are switching our participation to Thrust 1. We anticipate continued interactions with T2, particularly as Thrust 2 generates additional large-scale 'omics data. We will provide training as needed to T2 researchers (as well as other interested researchers) on the use of the PMR bioinformatics software and database we have developed and implemented for yeast.

Manuscripts are in preparation.

Expected Milestones and Deliverables

- Submit final RNA seq samples, complete data analysis and manuscripts.

Member Company Benefits

The yeast network database and model would provide an excellent tool for industry researchers in their analysis of factors that contribute to composition in relation to polyketide (acetate-based) test beds. Furthermore, we have identifies specific genes that impact lipid/polyketide composition in yeast. The genes identified in our analyses will facilitate systematic manipulation of flux and yield to target carbon flow to the desired polyketide compounds.

The PMR software developed can be applied to analysis of the effects of targeted manipulation of a wide range of compounds.

Commercialization / Technology Transfer

A provisional patent (no. 61/446.469) has been approved. The provisional patent, based on an associated NSF-funded project, is for a gene that controls accumulation of protein, carbohydrate and lipid in Arabidopsis and soybean.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.6A -** β-Oxidation Pathway Reversal in *E.coli*

Thrust: Research Thrust 2 - Microbial Metabolic Engineering

Pursuant to guidelines for preparing ERC annual reports, three- to five-page Project Summaries are required for all core and sponsored projects (those with direct support from the Center). A Project Summary should also be provided for each supplementary and special-purpose award received by the ERC. Project Summaries do not have to be included for proprietary projects where such a summary would compromise the sponsor's interests. In general, Project Summaries are NOT required for *associated* projects; rather, abstracts and other information for these projects will be collected separately. However, for Gen-3 ERCs, foreign partner associated projects may include a Project summary rather than only an abstract if the project is of particular importance to achieving the vision of the center.

Prepared By: **Ramon Gonzalez**	Date *(in U.S. date format)*: **02/14/13**	Reporting Period: **03/01/2012 to 02/28/2013**

ERC Team Members
Project Leader: Ramon Gonzalez, Rice University
Other Faculty: NA
Graduate Students: Maria Rodriguez-Moya, Seokjung Cheong, Rice University
Post-doctoral Scholars: James Clomburg, Jacob Vick, Matt Blankschien, Rice University

Statement of Project Goals
This project aims to further engineer the reverse β-oxidation cycle for the biosynthesis of ω-carboxylated products in *E. coli*.

Project's Role in Center's Strategic Plan
The results from this project will directly contribute to the carboxylic acids test bed.

Fundamental Barriers and Methodologies
This project could be limited, in general, by the ability to utilize the ω-carboxylated primers and react on ω-carboxylated intermediates by the reverse β-oxidation pathway.
Alternative enzyme selection and substitution and further metabolic engineering of the reverse β-oxidation pathway will be used for overcoming this barrier.

Achievements

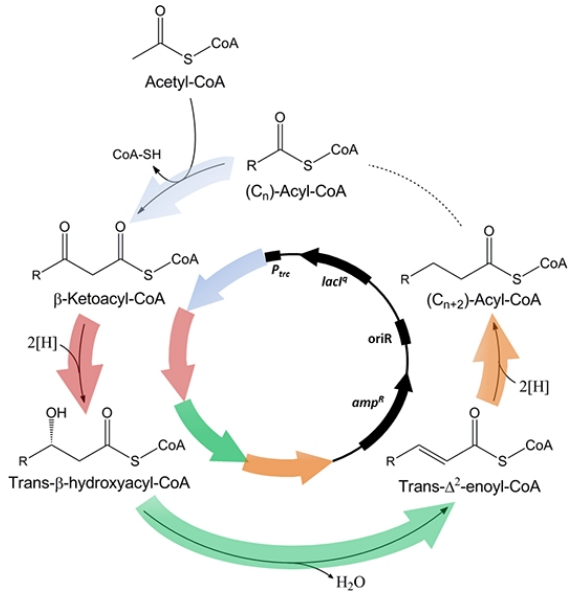


Figure 1. Engineered reversal of β-oxidation pathway is a valuable platform for efficient production of platform chemicals.

The introduction of the engineered reversal of the β-oxidation cycle (Figure 1) has opened many opportunities for engineering *E. coli* metabolism for the production of ω- and β-carboxylated products in an ATP-independent and efficient manner (Figure 2). Recent advances on this project have focused on characterizing the roles of β-oxidation enzymes (Table 1) and assembling pre-defined components *in vivo* conditions to enable production of a variety of carboxylic acids by combining different thioesterases. Production of acetate and butyrate (Figure 3), as well as production of short- and medium-chain fatty acids ranging from C_6-C_{12} (Figure 4) were achieved in this study. The characterization of β-oxidation enzymes via gene knockouts and overexpressions portrayed in these studies provided information about fatty acid oxidation enzymes that can power further efforts for engineering industrial microorganisms. More details about this study can be found in ***ACS Synthetic Biology*. 1(11): 541-554.2012.**

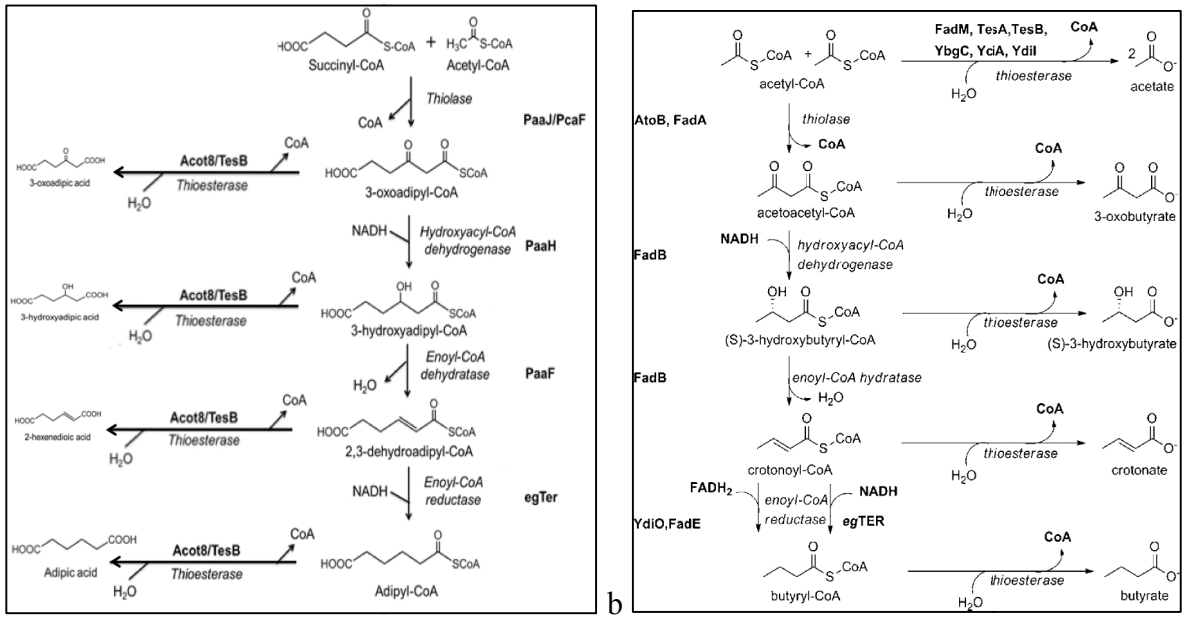


Figure 2. (a)One cycle of reverse β-oxidation pathway that produces ω-carboxylated products. This pathway priming from ω-carboxylated succinyl-CoA and terminated or branched out by dicarboxylic acyl-CoA thioesterase. After one cycle, adipic acid can be produced, and 3-oxoadipic acid, 3-hydroxyadipic acid and 2-hexenedioic acid can be yielded through branching-out from the pathway.
(b) One-turn functional reversal of the β-oxidation cycle with 4-C carboxylic acids as a proxy for product synthesis. A multiple turn cycle involves the condensation of the end acyl-CoA molecule (butyryl-CoA from a one-turn reversal) with acetyl-CoA resulting in a 2-carbon elongation.

Table1. In Vitro Kinetic Characterization of Enzymes Involved in a Functional Reversal of the β-Oxidation Cycle

enzyme	substrate[a]	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M (M^{-1} s^{-1})	specific activity (μmol mg^{-1} min^{-1})
AtoB	acetyl-CoA	3.17 ± 0.18	892.0 ± 56.5	(3.6 ± 0.3) × 10^3	0.919 ± 0.002[c]
	acetoacetyl-CoA	9.23 ± 0.34	68.1 ± 2.56	(1.36 ± 0.07) × 10^5	0.36 ± 0.05[c]
FadA	acetyl-CoA			7.5 ± 0.3[b]	not detected[d]
	acetoacetyl-CoA	0.75 ± 0.09	422.5 ± 57.3	(1.8 ± 0.3) × 10^3	0.013 ± 0.002[d]
FadB	acetoacetyl-CoA	25.9 ± 1.2	390.0 ± 19.2	(6.6 ± 0.4) × 10^4	0.185 ± 0.001[d]
	crotonyl-CoA			(3.2 ± 0.5) × 10^3[b]	0.051 ± 0.004[d]
egTER	crotonyl-CoA	1.14 ± 0.08	98.5 ± 7.7	(1.2 ± 0.1) × 10^4	5.4 ± 0.6[e]
FadE	butyryl-CoA				0.008 ± 0.001[c]
YdiO	butyryl-CoA				0.009 ± 0.003[c]
YciA	acetyl-CoA	5.9 ± 0.1	42.7 ± 1.2	(1.62 ± 0.05) × 10^5	0.222 ± 0.007[c]
	acetoacetyl-CoA	264.6 ± 54.7	875.7 ± 180.9	(3.0 ± 0.8) × 10^5	0.672 ± 0.007[c]
	RS-3-hydroxybutyryl-CoA	32.4 ± 3.5	353.8 ± 38.8	(9 ± 1) × 10^4	0.441 ± 0.009[c]
	crotonyl-CoA	46.4 ± 7.7	1348.8 ± 235.5	(3.4 ± 0.8) × 10^4	0.27 ± 0.03[c]
	butyryl-CoA	557.5 ± 79.6	641.8 ± 91.9	(9 ± 2) × 10^5	2.9 ± 0.2[c]

[a]Direction of assay indicated by substrate and enzyme as shown in Figure 1 [b]Could not be saturated. [c]Expressed from pCA24N. [d]Expressed from pUCBB-ntH6. [e]Expressed from pTrcHis2A.

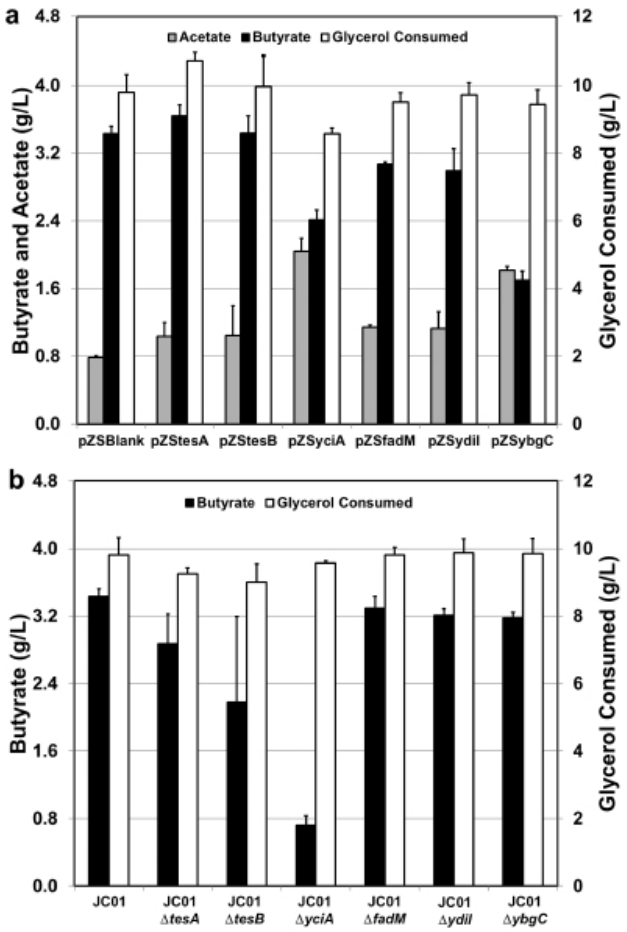


Figure 3. Termination pathway manipulation with a functional full one-turn β-oxidation reversal. (a) Glycerol consumption, butyrate, and acetate production in JC01 upon thiolase (AtoB), 3-hydroxyacyl-CoA dehydrogenase (FadB), enoyl-CoA hydratase (FadB), and trans-enoyl-CoA reductase (egTER) expression (i.e., pTHatoB.fadB.egTER) in combination with thioesterase overexpression via controllable construct. (b) Glycerol consumption and butyrate production in JC01 harboring pTHatoB.fadB.egTER and pZSBlank with native thioesterase chromosomal deletion. Gene overexpression apparent from plasmid names (i.e., pTHatoB.fadB.egTER expressing atoB, fadB, and egTER). Δ"gene" represents gene deletion.

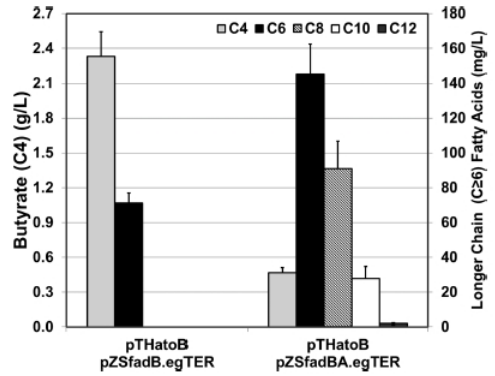


Figure 4. Operation of multiple cycle turns during a functional reversal of the β-oxidation cycle for the synthesis of longer-chain products. Distribution of extracellular fatty acid production by JC01 upon the expression of the functional units for a full one-turn reversal (pTHatoBpZSfadB.egTER) and with the inclusion of the long-chain specific thiolase FadA (pTHatoB pZSfadBA.egTER). C6, hexanoic (caproic) acid; C8, octanoic (caprylic) acid; C10, decanoic (capric) acid; C12, dodecanoic (lauric) acid.

Design of novel reverse β-oxidation cycle for synthesis of ω-carboxylated products

Engineered reversal of β-oxidation has been developed which is able to produce alcohols and carboxylic acids (Nature 476:355-359, 2011). The pathway starts from condensation of two molecules of acetyl-CoA, one as primer and the other as the donor of two carbons. Then, the carbon chain is extended through four enzymes of the reverse β-oxidation cycle: thiolase; 3-hydroxyacyl-CoA dehydrogenase; enoyl-CoA hydratase; enoyl-CoA reductase. Finally, the cycle is terminated or branched out by: i) thioesterase (for production of carboxylic acids); ii) acyl-CoA reductase and aldehyde/alcohol dehydrogenase (for production of alcohols). Due to lower ATP energy consumption and higher carbon yield, this pathway is regarded as the efficient platform for microbial production of advanced biofuels and chemicals.

However, currently the products of this developed reverse β-oxidation pathway are only functionalized in β group and there is no evidence showing that this pathway is capable of producing products with other functionalized parts, such as ω-carboxylated products. Since some of the ω-carboxylated products, such as adipic acid, the monomer of nylon-6,6, are with great values and their clean biosynthesis is required due to environmental and sustainability concerns, an ω-carboxylated products producing reverse β-oxidation pathway is needed.

To produce ω-carboxylated products, the enzymes of the pathway should be effective when reactants are ω-carboxylated. The enzymes of current reverse β-oxidation pathway are not known to react on them, so they are substituted with similar enzymes that are naturally effective on ω-carboxylated metabolites. Figure 1 shows the proposed one cycle of reverse β-oxidation for production of C_6 ω-carboxylated carboxylic acids. This pathway is composed of five steps: i) condensation of ω-carboxylated primer succinyl-CoA with 2C donor acetyl-CoA by β-ketoadipyl-CoA thiolase PaaJ from *E. coli* (PNAS 107: 14390-14395, 2010) or PcaF from *P. putida* (Journal of Bacteriology 184: 207-215, 2002), which degrades the 3-oxoadipyl-CoA to succinyl-CoA and acetyl-CoA; ii) hydrogenation of 3-oxoadipyl-CoA to 3-hydroxyadipyl-CoA by 3-hydroxyadipyl-CoA dehydrogenase PaaH from *E. coli* (PNAS 107: 14390-14395, 2010); iii) dehydration of 3-hydroxyadipyl-CoA to 2,3-dehydroadipyl-CoA by PaaF, the 2,3-dehydroadipyl-CoA hydratase in *E. coli* (PNAS 107: 14390-14395, 2010); iv) reduction of 2,3-dehydroadipyl-CoA to adipyl-CoA by egTer, an efficient enoyl-CoA reductase from *Euglena gracilis* which directly utilizes NADH as cofactor (Journal of Biological Chemistry 280:4329-4338, 2005); v) termination of the cycle, which yields adipic acid, or branching-out from the above steps, which yields 3-oxoadipic acid, 3-hydroxyadipic acid and 2-hexenedioic acid, respectively, by dicarboxylic acyl-CoA thioesterase Acot8 from mouse (Journal of Biological Chemistry 280:38125-38132, 2005), or by TesB, which belongs to same family with Acot8 according to ThYme database.

Except thiolases PaaJ and PcaF (Patent No. US20100317069) and Acot8 (Journal of Biological Chemistry 280:38125-38132, 2005), none of these enzymes have been reported to be able to catalyze the reactions as above. Therefore, their activity should be confirmed before the pathway is constructed. This will be performed mainly through *in vitro* reaction with analysis by spectrophotometric enzyme assay and LC-MS. *In vivo* fermentation in *E. coli* strain will also be operated to check whether the relevant ω-carboxylated acids are produced when these enzymes are overexpressed through cloning of genes in pTrcHis2A and pZS vectors. So far, the enzyme assays of PaaJ, PcaF and PaaH have been completed, and the other analysis is currently underway.

Enzyme assay results

PaaH (3-hydroxyadipyl-CoA dehydrogenase)

The synthetic activity of PaaH was performed through observation of NADH oxidation at 340nm. The enzyme was purified with Histidine-tag from ASKA library (National BioResource Project; DNA Research 12:291-299, 2005). Since the substrate 3-oxoadipyl-CoA is not available in commercial, acetoacetyl-CoA was used instead as the assay substrate. As in Table 2, the activity was observed, indicating that PaaH is also capable to hydrogenate 3-oxoadipyl-CoA, since PaaH naturally works on ω-carboxylated reactants.

Table 2. Enzyme assay results of *E. coli* 3-hydroxyadipyl-CoA dehydrogenase PaaH. The unit "U/mg" means "μmol/mg protein/min".

Enzyme	State	Origin	Substrate	Specific activity (U/mg)
PaaH	Crude	*E.coli*	acetoacetyl-CoA	1.03±0.06
PaaH	Purified	*E.coli*	acetoacetyl-CoA	3.10±0.22

PaaJ/PcaF (3-oxoadipyl-CoA thiolase)

After PaaH activity was confirmed, as in Table 3, by observing NADH oxidation at 340nm, the succinyl-CoA and acetyl-CoA condensation activities of PaaJ and PcaF were detected and measured through enzyme assay coupled with PaaH. PaaJ was purified with same way as PaaH, while PcaF was purified with His-tag from the *E. coli* strain harboring the codon optimized *pcaF* gene cloned in pTrcHis2A plasmid. The samples with only acetyl-CoA as substrate were used as control.

Table 3. Enzyme assay results of 3-oxoadipyl-CoA thiolases PaaJ and PcaF. "N. D" means "not detected".

Enzyme	State	Origin	Substrate	Specific activity (U/mg)
PaaJ	Purified	*E. coli*	Succinyl-CoA + acetyl-CoA	0.12±0.02
PaaJ	Purified	*E. coli*	Acetyl-CoA	N.D
PcaF	Purified	*Pseudomonas putida*	Acetyl-CoA	N.D
PcaF	Purified	*Pseudomonas putida*	Succinyl-CoA + acetyl-CoA	0.184±0.009

These results also indicate that PaaH is really capable of conversion of 3-oxoadipyl-CoA to 3-hydroxyadipyl-CoA. The PaaJ enzyme assay coupled with 3-hydroxybutyryl-CoA dehydrogenase HBD from *Clostridium acetobutylicum*, which can efficiently reduce acetoacetyl-CoA and 3-hydroxybutyryl-CoA (Nature Chemical Biology 7: 222-227, 2011), was also performed to probe whether HBD is effective on 3-oxoadipyl-CoA. This assay showed no activity of HBD.

In vitro LC-MS analysis

LC-MS analysis on *in vitro* enzymatic reactions of PaaJ and PaaH was also performed. Due to possibly low equilibrium constant of succinyl-CoA and acetyl-CoA condensation reaction, the product 3-oxoadipyl-CoA amount can be too low to be observed in sole PaaJ reaction system. The synthetic reaction activity of PaaH is relatively higher, so PaaJ was analyzed together with PaaH to drive the reaction continuously.

As Figure 5 shows, substrates succinyl-CoA and acetyl-CoA were consumed, and product HS-CoA was produced. However, unlike the expectation, the peak of target product, 3-hydroxyadipyl-CoA was not observed. Thus, more experiments and analysis on data are demanded to figure out whether the synthetic reactions of PaaJ and PaaH occur or not.

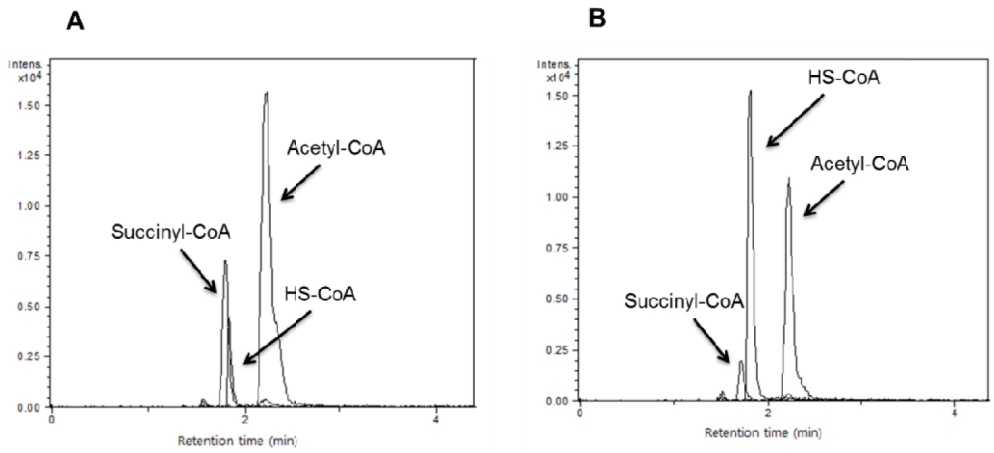


Figure 5. LC-MS ion chromatograms of acetyl-CoA (m/z=810.2), succinyl-CoA (m/z=868.2) and HS-CoA (m/z=768.1) after 0h (A) and 7h (B) of reactions of PaaJ and PaaH. The enzymatic reaction system volume is 100μL, composed of 100mM Tris-HCl (pH 7.8), 2mM succinyl-CoA, 1mM acetyl-CoA, 0.2mM NADH, 1mM dithiothreitol, 4.5mM $MgCl_2$ with 10μL of purified PaaH (~10μg) and 10μL of purified PaaJ (~5μg), and the reaction was performed under 30℃.

Other Relevant Work

Published manuscript:

Clomburg, J.M., Vick, J.E., Blankschien, M. D., Rodriguez-Moya, M., Gonzalez, R. (2012). A synthetic biology approach to engineer a functional reversal of the beta-oxidation cycle. *ACS Synthetic Biology*. 1(11): 541-554.

Plans for the Next Year

The confirmation of activities of the rest of enzymes of the pathway will be finished.
One cycle of ω-carboxylated products producing reverse β-oxidation will be constructed and optimized through metabolic engineering and other tools, such as protein engineering.

Expected Milestones and Deliverables

For next year we expect to:
Achieve synthesis of adipic acid through one cycle of engineered reverse β-oxidation.

Member Company Benefits

The implementation of an efficient modular pathway that produces ω-carboxylated acids and alcohols is expected to generate significant intellectual property, which in turn will benefit member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **02-12F1** - Student-led Research Grant: 13C Metabolic Flux Analysis-Based Fluxomics Comparison Between Saccharomyces cerevisiae and Oleaginous Yeast Yarrowia lipolytica

Thrust: **Thrust 2 - Microbial Metabolic Engineering**

Pursuant to guidelines for preparing ERC annual reports, three- to five-page summaries for all projects receiving direct support, organized by thrust and/or education/outreach program area, must be provided. Project summaries do not have to be included for proprietary projects where such a summary would compromise the sponsor's interests. A project summary should also be provided for each supplementary and special-purpose award received by the ERC. In general, project summaries are NOT required for *associated* projects; rather, only an abstract is required in these cases. However, for Gen-3 ERCs, foreign partner associated projects may include a project summary rather than only an abstract if the project is of particular importance to achieving the vision of the center. Each project summary should contain all the sections shown below. Figures, tables and charts are encouraged if they help to explain or validate findings and results.

Prepared By:	Date *(in U.S. date format)*: **03/31/2013**	Reporting Period: **03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Jacqueline Shanks, Dept of Chemical and Biological Engineering, ISU
Other Faculty:
Suzanne Sandmeyer, Department of Biological Chemistry, UCI
Graduate Students:
Ting Wei Tee, Department of Chemical and Biological Engineering, ISU
James Yu, Department of Biological Chemistry, UCI
Postdoctoral Researchers:
Ivan Chang, Department of Biological Chemistry, UCI

Statement of Project Goals

The goal of the project is to construct metabolic flux maps for *Yarrowia lipolytica*, for both the wild-type and engineered strains and under various operating conditions. The flux maps from this project will be used to guide further genetic manipulations for strain improvement.

Project's Role in Center's Strategic Plan

13C metabolic flux analysis (MFA) provides a realistic in vivo profile of metabolic flux distributions (fluxomics) of an organism by relying on the 13C carbon tracing patterns as additional constraints rather than a growth objective function typical in flux balance analysis (FBA). This project extends the MFA expertise available at the CBiRC Center to branch site in UC Irvine for the purpose of obtaining fluxomics of the industrially relevant oleaginous yeast *Yarrowia lipolytica*. Unlike *Saccharomyces cerevisiae* which stores its energy as polysaccharide, oleaginous yeasts store energy as lipids and may therefore have enhanced potential for production of industrially desirable compounds. The outcome would enable valuable comparisons between the yeast systems studied on the two campuses and provide greater basis for Center collaborations.

Fundamental Barriers and Methodologies

A key overall goal of Thrust 2 is to construct strains that enhance production of carboxylic acids and pyrones. *Y. lipolytica*, an oleaginous yeast, can accumulate up to 70% of its biomass in lipids, making it an attractive testbed organism for strain development. Fundamental barriers for performing 13C MFA on *Y. lipolytica* are that 1) the growth characteristics in *Y. lipolytica* are significantly different from *S. cerevisiae*; 2) the metabolic pathway of *Y. lipolytica* is less well known, leading to difficulties constructing the required MFA model; 3) the branch site has no dedicated GC-MS or HPLC machines for quantitative measurement of metabolites in MFA; 4) outcomes in the 13C MFA results are highly sensitive to the metabolic model and computational methods used in the analysis.

For the determination of the growth rate, growth curves for wild type and Δ*gut2* mutant *Y. lipolytica* were constructed (see Achievements). For the expertise and experience, graduate student Ting Wei Tee joined the UCI branch site for two weeks in February, and one week in March to participate in the development of the *Y. lipolytica* specific 13C MFA protocol, and execution of the 13C MFA experiment using the tools and instruments available at UCI. For computational analysis of 13C MFA experiment results, MFA computation software, *13CFlux2* and *FiatFlux*, were used to cross-compare and assess modeling consistency. In addition, visualizations of the resulting fluxomics data are carried out using the pathway editing software *Omix*.

Achievements

1. We have characterized the growth properties and lipid production of *Y.lipolytica* under carbon and nitrogen limitation conditions. A growth curve for both of these conditions was constructed (Figure 1a). Lipid production was measured for cells in log, late log, early stationary, and late stationary. In late stationary, nitrogen-limited cultures produced four-fold greater lipid than carbon-limited cells. The major fatty acid was C18:1, with second highest C18:2 in both carbon- and nitrogen-limited growth. This reflected greater than 20% fatty acid per gram dry weight in the nitrogen-limited sample (Figure 1b).

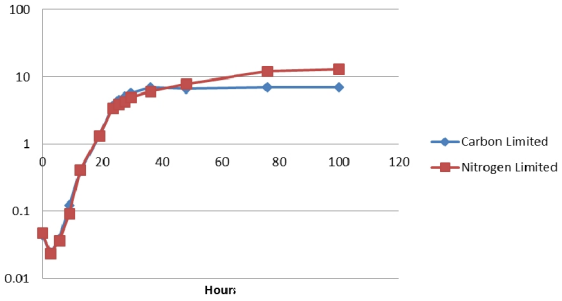


Figure 1a. Y.lipolytica Growth Curve

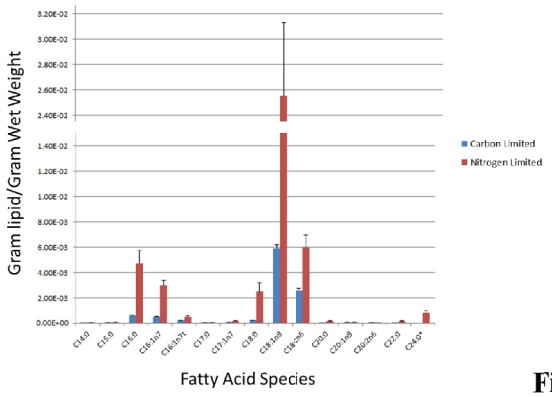


Figure 1b. Lipid production by fatty acid species

2. The gene for the mitochondrial glycerol-3-phosphate dehydrogenase (*gut2*) was previously deleted and the resulting *gut2*Δ mutant was also characterized. The gene for mitochondrial glycerol-3-phosphate dehydrogenase was deleted using *URA3*, flanked upstream and downstream by 1000bp of *gut2* flanking sequence, selected on ura- medium, screened on glycerol for no growth, and confirmed by PCR (data not shown). This strain was grown under nitrogen-limiting conditions and unsaturated lipids were evaluated using the phospho-vanillin assay .

3. We have characterized a cellular morphology difference between *WT* and *gut2Δ Y.lipolytica* in stationary phase. The *gut2Δ* mutant maintains a high percentage of filamentous cells compared to the budding cells in the WT (we suspect a link with its elevated lipid production).

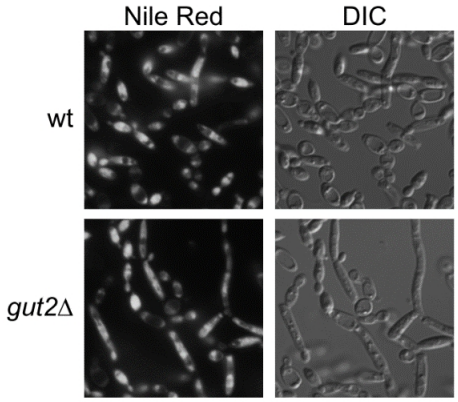


Figure 2. Dimorphism in *Y. lipolytica*. Morphology of WT vs *gut2Δ* in early stationary phase via differential interference contrast microscopy. Nile red dye stains for intracellular lipid droplets. Images provided by Virginia Bilanc of the Sandmeyer lab.

4. We have performed the 13C MFA experiment at UCI on both WT and *gut2*Δ *Y. lipolytica* using a combination of 20% uniformly labeled 13C glucose and 80% 1-13C labeled dextrose as the only carbon tracers. Extracellular measurements of metabolites were profiled using HPLC, glucose uptake rate was obtained via glucose assay kit, and fatty acid production was quantified using GC-MS (data incorporated in the model). The isotopomer fraction of proteinogenic amino acids and quantified using both the student open access GC-MS machine at UCI chemistry facility and the dedicated GC-MS machine at ISU. We have identified and sorted out the variously technological and logistical issues in performing the *Y. lipolytica* 13C MFA experiment at UCI and modified the experiment protocol to adapt to the instruments available to produce robust fluxomics results.

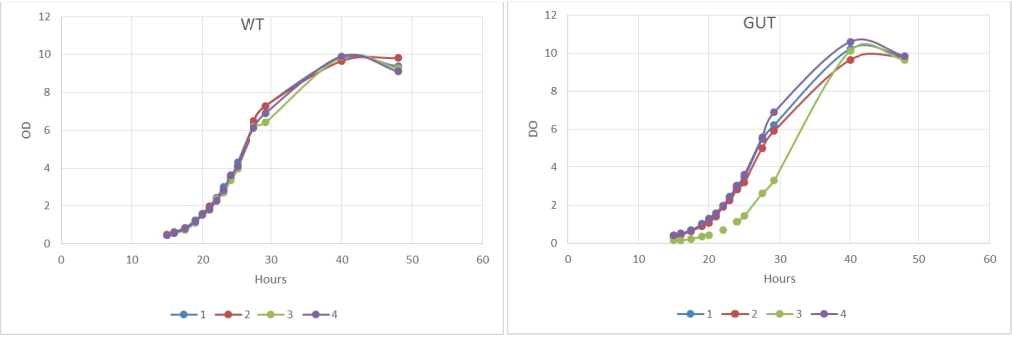


Figure 3. Growth of *Y. lipolytica* WT and *gut2*Δ (four replicates each) during the 13C MFA

5. We have successfully constructed a data consistent metabolic model of *Y. lipolytica* for 13C MFA via multiple rounds of iterative modifications to the metabolic model of the previous *S.*

cerevisiae 13C MFA (Figure 4a). The resulting fluxomics from the carbon tracing simulations via 13CFlux2 are then mapped to the model using Omix (Figure 4b). The flux distributions between *Y. lipolytica* and *gut2*Δ are similar at carbon-limited condition at exponential growth phase. Difference in flux distributions might be observed under nitrogen limited environment where *gut2*Δ mutant produced more lipids than the wild type. Most of the carbon fluxes (around 83%) channel through pentose phosphate pathway to generate NADPH for reduction requirements. The remaining carbon flux (around 13%) is directed towards the glycolytic pathway, resulting in low activities of the lower glycolytic pathway. The TCA cycle operates at 57 mmol $gDW^{-1}\,h^{-1}$ (based on 100 mmol $gDW^{-1}\,h^{-1}$) to generate ATP and NAD(P)H for energy and reduction requirements for cell growth. The glyoxylate cycle is active with 15 mmol $gDW^{-1}\,h^{-1}$ to bypass the TCA cycle. Anaplerotic reactions (phosphoenolpyruvate carboxylkinase and malic enzyme reactions) are not significant.

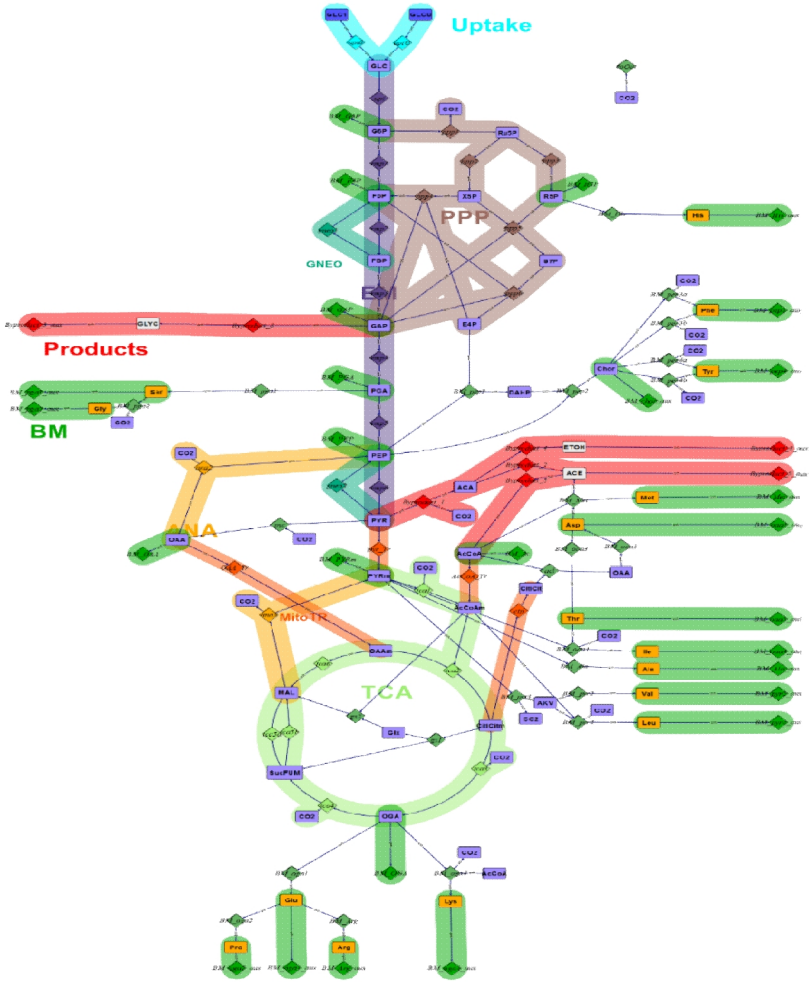


Figure 4a. Metabolic Pathway Model of *Yarrowia lipolytica*. Model was constructed in *Omix* for use in 13C MFA carbon tracing simulation. Metabolites are shown as rectangular boxes, with blue representing carbon source, purple - intermediate metabolites, white - potential extracellular metabolite products, and orange - amino acids. Reactions are shown as diamond shapes, and are grouped into sub-pathways, with cyan representing substrate uptake, purple – glycolysis pathway, aqua green – gluconeogenesis, brown – pentose phosphate pathway, light orange – anaplerotic pathway, red orange – transport to mitochondria, light green – TCA cycle pathway, green – reaction going into biomass, and red – exchange with media.

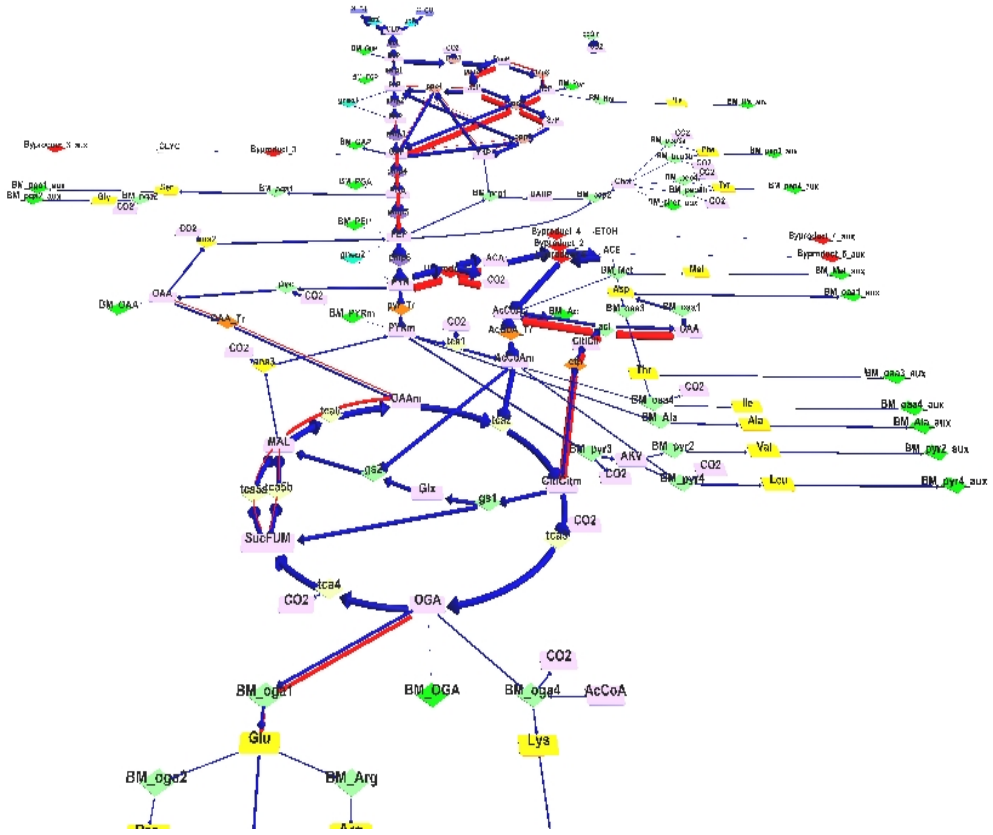


Figure 4b. Fluxomics of WT *Yarrowia lipolytica* (displayed in 3D via *Omix*). Fluxomics representing the optimal simulation of WT *Y. lipolytica* metabolic fluxes as constrained by the mass fraction labeling patterns derived from the 13C carbon tracing. Blue arrow edges representing forward fluxes, while red arrow edges representing reverse fluxes. The size of the edges are displayed in log scale.

Other Relevant Work

Flux analysis methods in yeast and plant systems leverage flux tool development for *E. coli*.

Plans for the Next Six Months

Integration of fluxomic data and existing RNASeq transcriptomic data of WT *Yarrowia lipolytica* into the hybrid kinetics-FBA framework of the genome-scale reconstructed model of *Yarrowia lipolytica*. RNASeq experiment will be performed on the *gut2* mutant to supplement existing data on WT *Y.lipolytica*.

Expected Milestones and Deliverables

1. Fluxomic data of WT *Yarrowia lipolytica* under specific growth conditions such as limiting nitrogen where fatty acid is supposedly increased.
2. Fluxomic data of *gut2*Δ*Yarrowia lipolytica*.
3. Fluxomic data of *Yarrowia lipolytica* under the effect of ATP citrate lyase (ACL) gene under/overexpression where the difference in citrate metabolism may show comparative advantage to *Saccharomyces cerevisiae* in fatty acid production.
4. Integration of 13C MFA fluxomic data and RNASeq transcriptomic data into the hybrid kinetics-FBA framework of the genome-scale reconstructed model of *Yarrowia lipolytica* as the basis for future *in silico* studies on *Yarrowia lipolytica* metabolism.

Member Company Benefits

Yarrowia lipolytica is an industrial-relevantyeast due to its favorable metabolic properties related to its oleaginousity. Many CBiRC member companies have shown interest in working with this particular yeast strain but do not have the resources for detailed analysis of its metabolic capabilities. Our team has extensive experience in experimental metabolic flux analysis, constructing genome-scale metabolic models and computational metabolic flux analysis and optimization procedures. The integrated flux platform is being demonstrated in CBiRC testbeds, and will create a powerful tool for strain design and optimization.

Commercialization / Technology Transfer

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **32-12F2** - Functional Genomics Profiling of the Oleaginous Yeast Stress Response

Thrust: Research Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*: **02/28/2013**	Reporting Period: **03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Suzanne B. Sandmeyer, University of California – Irvine

Postdoc: Ivan Chang, University of California – Irvine

Graduate Student: and James Yu, University of California – Irvine

Other Personnel: Brandon Haghverdian, University of California – Irvine

Statement of Project Goals

A major fraction of most fungal genomes have no known function. The model yeast *Saccharomyces cerevisiae* (Sc) provides one of the most compelling examples of the utility of genomewide approaches in addressing these unexplored genes. Arguably one of the most strategic resources is a collection of strains, each member of which was individually deleted for a nonessential open reading frame (~4500/6000)(Goffeau et al., 1996). This knockout (KO) collection (Winzeler et al., 1999) has been screened individually or as a pool to predict testable functions for thousands of genes (Giaever et al., 2002). Unfortunately a systematic deletion collection is not available for most industrial yeasts, among them the oleaginous strain *Yarrowia lipolytica* (*Yl*). The goal of this seed grant is development of a transposon profiling system for *Yl* to enable functional genomics. An important application is identification of genes which are required for growth under industrial "stress" conditions.

Project's Role in Center's Strategic Plan

Goals of the CBiRC project in the Sandmeyer laboratory include the development of molecular tools for *Yl*, modification of *Yl* for testbed compound pyrone synthase expression (Jez et al., 2000), and development of a hybrid (flux-kinetic) model. A *Yl*TEF1 promoter (Muller et al., 1998) has been introduced into a *Yl*URA3-marked expression plasmid upstream of GFP (unpublished data) and shown to be active by fluorescence activated cell sorting (FACS) analysis of *Yl* transformed with these plasmids. The base plasmid was patterned on our combinatorial *Sc* plasmids (Fang et al., 2010). The Sandmeyer laboratory has previously recoded domains from the Ty3 retrotransposon and other proteins for expression in *Sc* and *Escherichia coli* [e.g.(Larsen et al., 2008)]. In addition to experience with *Yl*, Sandmeyer is experienced in application of Illumina sequencing to transposon profiling (Qi et al., 2012) and is Director of the High-throughput Genomics Facility at UCI (http://dmaf.biochem.uci.edu/).

Fundamental Barriers and Methodologies

Oleaginous yeasts have advantages for biosustainable industrial applications because they accumulate a high percentage of cell weight as polyunsaturated lipid and grow readily on industrial by-product carbon sources such as acetic acid and glycerol (Ageitos et al., 2011; Beopoulos et al., 2011). Yl is one of the better characterized oleaginous yeasts at the genomic level. For example, an annotated sequence of the genome is available (Dujon et al., 2004) (http://www.genolevures.org/yeastgenomes.html#). Although it is relatively easy to manipulate from a molecular perspective, many of the tools available in Sc are lacking. Out of approximately 6500 ORFs in the *Yl* genome about 300 have a known function, 1000 cannot be assigned a function, and functions can be only be inferred for the remainder based on homology with genes from other yeast (T. Najdi, unpublished data) (http://www.genolevures.org/yeastgenomes.html#). Nonetheless, *Sc* is an imperfect model for *Yl* since it is only distantly related and has differences in gene expression as well as metabolism with *Yl*. For example, more extensive splicing occurs in *Yl* than *Sc*, and alternative splicing has recently been implicated in *Yl* transcriptome regulation (Kabran et al., 2012).

Despite its value, the *Sc* deletion collection represents an enormous investment of resources. Another approach to functional profiling relies on random transposon mutagenesis of target genomes (Smith et al., 1996). High-throughput sequencing by enabling rapid identification of transposon insertion sites (Guo and Levin, 2010) has revolutionized transposon profiling. The three key steps of this approach are: 1) mobilization of a tagged sequence by transposase expression; 2) selection of cells which have undergone transposition; 3) growth of cells under some condition of interest (e.g. stress); and 4) comparison of insertion site profile by high-throughput sequencing before and after exposure to the condition of interest. Given that the original pattern is relatively random and there is statistically "deep" coverage, genes which no insertions or only truncating insertions are concluded to be essential or growth rate limiting. This approach is: 1) economical; 2) relatively rapid; 3) informative on essential genes and potentially alternative splicing products; and 4) potentially enables mobilization by transposition of cis-acting sequences for strain evolution. We propose to use a hAT DNA cut-and-paste DNA transposon as this family is represented among a broad range of phylogenies (Arensburger et al., 2011).

Foreign Collaborations

Not applicable.

Achievements

This project was only recently funded (mid-February, 2013) through the Center's IAB Seed Funding program. Consequently, there are no accomplishments to report at this time.

Plans for the Coming Period

1. Construct a Hermes expression plasmid for expression in Yl.

The Hermes hAT element expression system in *Schizosaccharomyces pombe* (*Sp*) has been described in detail (Evertts et al., 2007; Park et al., 2009) and a similar system was used in *Sc* (Gangadharan et al., 2010); we will follow the broad outlines of those protocols. The entire Hermes element consists of a 2749 bp segment of DNA encoding the Hermes transposase (a sequence specific type of DNA recombinase) flanked by inverted repeat sequences that are the substrate for the transposase (Zhou et al., 2004). Briefly, this enzyme cleaves at the inverted repeat ends of the

2749 bp DNA and mediates transfer of the DNA to a new site in the genome. For purposes of transposon mutagenesis, it is desirable to separate the trans-acting transposase function from the mobilized sequence with Hermes inverted repeat ends. If, for unanticipated reasons, Hermes is not active, we will attempt to derive a superactive mutant and as a fall back will adopt a similar approach using Sleeping Beauty (Ivics and Izsvak, 2005), which is also broadly active in heterologous organisms.

a. *Expression of Hermes in Yl.* We will acquire from Dr. Levin (NIH), the Hermes element which was successful in *Sp* (Park et al., 2009) and express an HA epitope-tagged version of the transposase under the *Yl*TEF1 promoter (Muller et al., 1998) on a *Yl*LEU2-marked plasmid in a ura3 leu2KO *Yl* CLIB89 mutant. Expression will be tested by immunoblot analysis and compared to HA-tagged *Yl*PGK1 expressed from the endogenous locus. If expression is low, standard strategies will be used to improve expression. If RNA levels are problematic, we will test the effect of tandem upstream activating sequence (UAS) (Blazeck et al., 2011); if expression at the protein level is low, we will recode the transposase gene using *Yl* codon tables (http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=284591). If expression remains problematic, we will design and test a high-copy plasmid based on the *Sc* 2 micron vector (Volkert and Broach, 1986). If protein is made we will simultaneously test transposition activity as described below.

b. *Modifications to improve expression and transposition frequency as necessary.* A hygromycin resistance (HygR) gene will be cloned flanked by Hermes inverted repeat sequences on a URA3-marked plasmid. The *Yl*URA3-marked HygR and *Yl*LEU2-marked transposase plasmid will be transformed into *Yl* and transformants selected and grown in –Leu, -Ura synthetic dextrose (SD), basically as described for *Sc* and *Sp* transposon mutagenesis (Evertts et al., 2007; Gangadharan et al., 2010). Cells will be evaluated for transposition based on recovery of HygR cells which are 5FOA resistant indicating they have lost the *Yl*URA3-marked plasmid (Boeke et al., 1987), but undergone insertion of the HygR marker into the genome. HygR, 5FOA-resistant cells will be screened for the frequency of His- and ade2- cells compared to the starting population. If transposon-based mutagenesis is successful based on the number of possible target genes, background mutation rate and anticipated several percent frequency of single transpositions, we anticipate an order of magnitude increase in frequency of a particular auxotroph or in the case of ade2 (YAL010B231881p) disruption, red color. If the frequency of transposition is unacceptably low and we are satisfied that expression is at the expected level, we will explore an alternative plan including identifying hyperactive Hermes elements and another cut-and-paste element such as Sleeping Beauty (Ivics and Izsvak, 2005).

2. Transposon mutagenesis of Yl CLIB89

a. *Transposition.* Transformed cells will be grown and allowed to undergo transposition in several independent cultures. Cells containing transposed elements which have lost the plasmid will be selected and grown under nonselective conditions to expand the culture. A large amount of culture will be frozen for future use. DNA from the transposed cells which have lost the plasmid will be sequenced using protocols developed for mapping retrovirus and retrotransposon insertion sites. Because of the bias against nucleosome-occupied regions, we anticipate requiring similar numbers of insertions as were obtained in the *Sc* and *Sp* studies with Hermes (~200,000 insertion sites).

b. *Illumina next generation sequencing of insertion sites.* This approach specifically amplifies

insertion junctions so that a large number of insertions are sequenced very efficiently. Basically DNA is sheared to approximately 200 bp, and adapters with recessed blocked 3' ends are ligated to fragments. Primers complementary to Illumina sequencing primers and specific for the transposon sequence and for the adapter are used to further amplify the DNA. Since only transposon sequence is amplified in the first round, it insures that only adapters opposing a transposon sequence can be priming sites in the second reaction [e.g. (Guo and Levin, 2010; Qi et al., 2012)]. DNA fragments are quantified by qPCR and loaded onto the cBot cluster station and subsequently onto the Illumina HiSeq2000 for sequencing. Because approximately 200 million reads are recovered one lane of 72 cycle sequencing and the genome is relatively small, this is sufficient for triplicates of several experiments.

c. *Analysis.* Images are converted to base calls through the Illumina ELAND pipeline and downloaded as fastq files from which sequences are aligned to the *Yl* reference genome. At this point we will evaluate average depth of coverage of the genome and determine statistically whether coverage is sufficiently deep to conclude that genes which lack insertions are essential under the initial conditions. If this is not the case, we will proceed to collect additional samples. Whether replicas are consistently identifying common genes will be determined using approaches (e.g. Tuxedo suite) developed for ChIP-seq and RNA-seq (Pepke et al., 2009).

3. Transposon profiling to identify genes which are important for stress conditions

a. *Growth of strains under stress conditions.* If depth of coverage is sufficient to identify essential genes, we will generate a differential map of insertions of pooled transposed populations grown under "nonselective" conditions and conditions expected to enrich for specific functions. These conditions will include nitrogen limitation and replete carbon, a condition which favors lipid accumulation in oleaginous strains and resistance to starvation (Beopoulos et al., 2008). Based on experience of the Levin laboratory with *Sp*, we assume approximately 80 generations or less than one week would be required to obtain a population which has significantly fewer members with 10% or more difference in growth rate. Populations would be sampled over time to test this assumption and number of generations adjusted as necessary. Sequence analysis of these cultures would follow a similar regime to that described in 2. The two sets of sequences would be compared for a differential profile identifying genes likely to be important under the restrictive condition.

b. *Enhancer trap evolution of stress resistant strains.* If we are successful at disruption mutagenesis, we would develop a mobile UAS and use it to transpose activation into the *Yl* genome. For proof of principle, we would identify cells that survive better at elevated temperatures. This is a property that would be potentially beneficial for industrial strains.

Expected Milestones and Deliverables

Backup strategies are presented, but the following timeline assumes it is not necessary to switch to another transposon:

a. Strains and plasmids: Production of ura3/leu2 prototroph for Hermes expression: Q1

b. Expression testing and optimization of Hermes transposition: Q2-4

c. Sequence analysis and pooled transposed populations of cells: Q3-4

d. Differential profiling under "selective" stress and differential profiling and selected pool: Q4 into renewal year

Member Company Benefits/Commercialization Impacts

a. Tools: Development of a transposon mutagenesis system for *Yl* would demonstrate utility of this system in oleaginous yeast for the first time. Two specific tools would be: 1) an element optimized for expression and activity available for proprietary applications; and 2) a complex pool of mutagenized cells which could be grown under selective conditions of interest to companies or individual labs and then sequenced. These tools would be useful to the Sandmeyer laboratory working on *Yl* and would indirectly benefit collaborating academic laboratories (Da Silva, Noel, Shanks) and CBiRC corporate partners working with *Yl* and potentially working with other poorly-characterized industrial strains.

b. Findings: Functional profiling would be expected to: 1) identify essential genes under our "nonselective growth conditions" possibly to include splice variants; 2) genes which improve growth or survival under specific stress conditions; and 3) genes which are essential under specific stress conditions. Beneficiaries are as above.

c. Grant support: Work will provide preliminary data for an NSF *Yl* functional genomics grant application.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.2 -** Selective Dehydration of Model Compounds

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Brent Shanks	**02/13/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Brent Shanks, Iowa State University

Collaborator: James Dumesic, University of Wisconsin-Madison, George Kraus, Iowa State University, Matt Neurock, University of Virginia

Graduate Students: Michael Nolan, Tianfu Wang, Uma Wanninayake, and Anita Bejile, Iowa State University, Qiaohua Tan, University of Virginia

Undergraduate Students: Justin Glasper, Elliot Combs, Geng Sun, and Dalton Hughes, Iowa State University

Statement of Project Goals

Biorenewable feedstocks have excess oxygen relative to the amount typically present in industrial chemicals. Dehydration is an important reaction for the removal of oxygen, but limited work has been performed on selective dehydration in the presence of additional functionality in the reactant. An important goal in developing a catalytic "tool chest" for biorenewable chemicals will be demonstration of effective selective dehydration catalysts. In order to rationally design and develop more effective dehydration catalysts, it is also important to investigate the underlining structure-reactivity relationships among different catalysts that have been identified to effective.

Project's Role in Center's Strategic Plan

Selective dehydration in general will be an important chemical catalyst capability in the center. In this context, two different types of molecules have been chosen as model dehydration reactions to probe the intrinsic catalytic properties of various catalysts. The first one is to study selective dehydration catalysts that will be necessary for successful development of 1,6-hexanediol production; and the second one focuses on the understanding of selective catalytic dehydration of C6 sugars to produce 5-hydroxylmethylfurfural (HMF).

Fundamental Barriers and Methodologies

1.Selective dehydration of 1,2,6-hexanetriol

The fundamental selectivity barrier in selective dehydration of polyols is the selective formation of linear species relative to ring-shaped species. Typical results for acid-catalyzed dehydration of 1,2,6-hexanetriol has routinely favored selectivity to pyran ring products over the desired linear products. New understanding over the last year has found that the main selectivity driver appears to be the activated species in the dehydration reaction, and

manipulation of reaction kinetics could enhance selectivity to linear species.

2.Selective glucose dehydration to HMF
The major hurdle that limits the high yield of glucose dehydration to produce HMF is the instability of HMF in the relatively harsh reaction media which is required to dehydrate glucose. In this context, a biphasic system consisting of an reactive aqueous phase, where the glucose is dehydrated catalyzed by various Lewis acidic metal saltes and an organic phase which could continuously extract the produced HMF, has been demonstrated to be an effective catalytic system to obtain high yield of HMF. Since last year, a systematic study has been performed to study the effect on catalytic activities of reaction conditions such as solution pH values, reaction temperatures etc. New insights on how these parameters could affect the catalytic activities could aid the rational design of Lewis acids-based catalysts for glucose dehydration.

Achievements

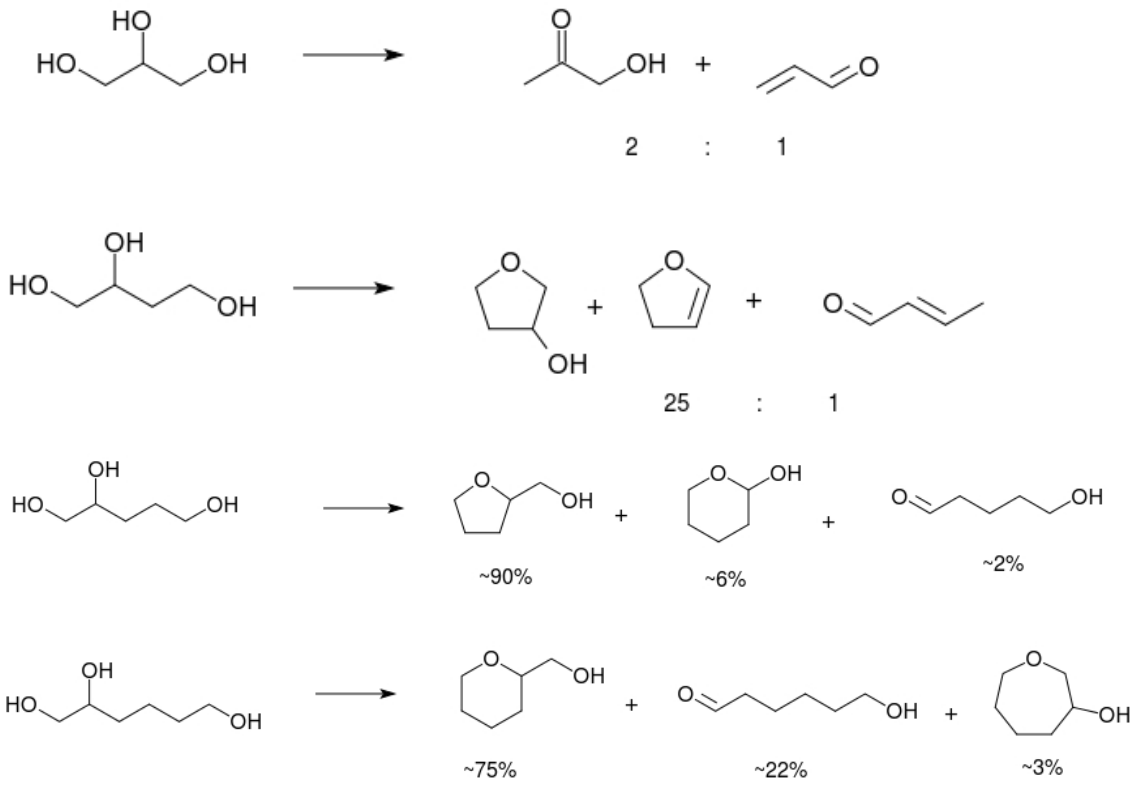
1.Selective dehydration of 1,2,6-hexanetriol
Selective dehydration of 1,2,6-hexanetriol – reaction pathway and mechanisms

Continued investigation of the dehydration pathways of 1,2,6-hexanetriol have found that the triol undergoes both acid-catalyzed dehydration and MPV-type hydride transfers over aluminosilicates. The formation of 6-hydroxyhexanal has been observed as a major dehydration product, and is proposed to be the primary source of hydride transfer products, namely 1,6-hexanediol (MPV reduction) along with caprolactone and tetrahydropyran-2-formaldehyde (Oppenauer oxidation). Coordination of alcohols and and aldehyde bonds over alumina sites are proposed to be the primary driver of these hydride transfers.

Further mass spectral studies have found that condensation products observed during the dehydration reaction are derived from linear products, which was inferred from an absence of signals corresponding to pyrans. Aldol condensation of 6-hydroxyhexanal with itself and other molecules is proposed to be the cause, highlighting again that the introduction of an aldehyde in the reaction scheme opens up new reaction pathways in polyol dehydration.

Dehydration of additional triols

Reaction studies of glycerol, 1,2,4-butanetriol, and 1,2,5-pentanetriol have been carried out over amorphous silica-alumina to determine the effect of chain length. The general trend was for short molecules (3- and 4-carbon) to favor dehydration of primary hydroxyls, and for longer chain triols to dehydrate internal hydroxyls. For glycerol, all products were linear in nature. For the butanetriol, the primary products were furans, indicating that ring-closing by dehydrating a primary hydroxyl was favored over linear elimination, with an average ring/linear ratio of 26:1. For pentanetriol and hexanetriol, ring formation by dehydrating the internal hydroxyl was favored over forming a hydroxyaldehyde, though for pentanetriol the average ring/linear ratio was 84:1, whereas for hexanetriol it was 3:1. The dropoff in ring selectivity suggests that ring size is a strong indicator of ring formation, though the mechanism for this relationship is not yet clear.



2.Selective glucose dehydration to HMF

2.1. pH effects of pH values on Lewis acidity of different metal salts

The Lewis acidity of different metal salts is a function of both the metal concentration and the solution pH values. Typically, the metal exists in an aqu ion form under low pH conditions while gradually changing into polyhedron and metal hydroxide forms under higher pH values. Depending on the nature of the cations, they could be categorized into water-sensitive and water-stable Lewis acids. As shown in the figure below entailing the kinetic profile of different Lewis acids, for the water-sensitive metal salt, $AlCl_3$, the initial rate dropped significantly when the solution pH was changed from 2.5 to 5.5; however, for the water-sable Lewis acid $YbCl_3$, the initial rate was comparable under different pH values studied. This difference could be explained from the different hydrolysis behavior of the two metal salts with $YbCl_3$ being the more water-stable Lewis acid.

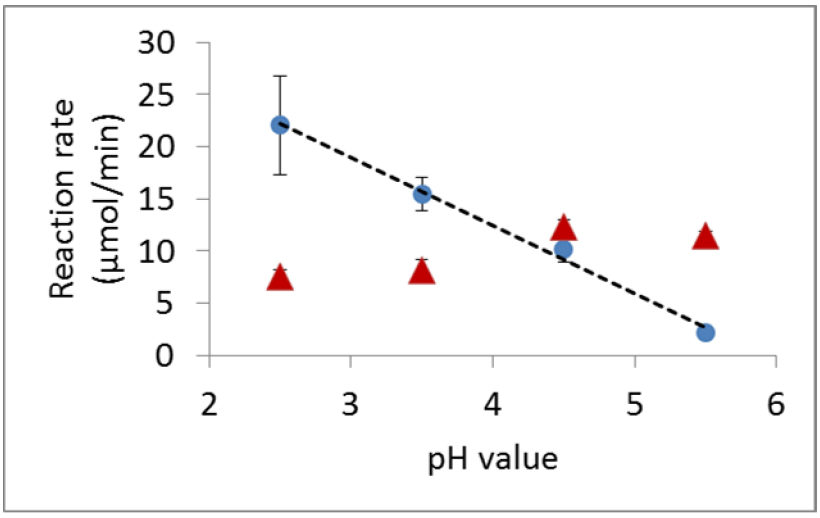


Figure: Effect of solution pH value on the catalyst activities (Δ $YbCl_3$; O $AlCl_3$)

2.2. Activation energy studies of different metal salts

A more comprehensive activation energy studies with different metal salts were also carried out as shown in the table. Taking the reaction temperature of 160 °C as an example, the reaction rate constant decreases from 669 for $AlCl_3$ to 65 for $InCl_3$. The decreased reactivity of In compared to Al might be explained by the larger cation radius of In, likely resulting in weaker metal-glucose interaction. The same trend was also observed for the lanthanide series. While comparing the reaction rate at a single temperature for different metal gave qualitative information regarding different metals, studying the activation energies over the selected temperature range is necessary to compare the intrinsic activities of the Lewis acids.

Table: Rate constants and activation energies for different Lewis acids

T, °C	$AlCl_3$	$GaCl_3$	$InCl_3$	$YbCl_3$	$DyCl_3$	$LaCl_3$
150	316±4	52.8±2.1	32.5±0.7	81.2±2.1	37.8±1.2	11.7±0.5
160	669±19	86±0.7	64.5±7.3	132.8±4.7	99.3	42.6±1.6
170	1729±39	175±15	173±36	248.3±13	180±9	102.9±1.0
Ea (kJ/mol)	125±3	93±3.4	128±18	87±2.5	121±2	169±10

Other Relevant Work

The results for 1,2,6 hexanetriol dehydration are not only important for understanding selective dehydration of polyols, but also connects with work in selective oxidation and selective ring opening. Managing hydride transfer reactions may work in tandem with dehydration to produce a broader array of molecules from a given substrate.

In order to gain more information about the glucose dehydration information, 2-D labeled glucose and normal 2-H glucose dehydration kinetic profile with the same reaction condition were compared and clearly, 2-D glucose had a much slower conversion rate, indicating the 2-D is involved in the

rate-limiting step in the proposed reaction mechanism. This finding is consistent with the work reported by others using heterogeneous zeolite-based catalysts.

Plans for the Next Year

Dehydration of 1,3,ω triols

Dehydration studies have focused primarily on situations in which there are two neighboring hydroxyls. The next set of molecules for study will include triols that do not have neighboring hydroxyls, in order to study mechanistic changes.

Bifunctional Catalysis

Preliminary results from studies of dehydration over metal-acid bifunctional catlaysts has found favorable selectivity toward linear products, but ring-opening of pyrans does not account for the enhanced selectivity. Additional testing is being carried out to discover the mechanistic difference between the bifunctional catalysts versus using aluminosilicate supports only.

Supported Lewis acids for glucose dehydration

Hydrothermally stable carbon based sulfonates will be synthesized and characterized as support to immobilize Ln as solid Lewis acid to perform the glucose dehydration reaction. The use of solid acid benefits from the ease of catalyst separation and possibly for the continuous production of HMF with a flow reactor system.

Expected Milestones and Deliverables

Testing of metal-acid bifunctional catalysts will be carried out with 1,2,6-hexnetriol, HMTHP (selective ring opening) and 1,6-hexanediol (selective dehydrogenation to 6-hydroxyhexanal, and upgrading to caprolactone). 1,3,6-hexanetriol and 1,3,5-pentanetriol will be synthesized as part of a collaborative effort, and tested over aluminosilicates for comparison to results from dehydration of 1,2,ω triols.

Several different methodologies will be exploited to synthesize hydrothermally stable carbon based supports. Once synthesized and optimized, the Ln will be supported onto these solids to generate heterogeneous Lewis acids. Reaction testing will be performed, focusing on the selection of the solvent in which the reaction is taking place as well as optimizing the reaction parameter to maximize the HMF yield.

Member Company Benefits

Discovering a complete set of dehydration rules for polyols will broaden the array of attractive molecules that can be derived from dehydration reactions. Solid Lewis acid for glucose dehydration could help ease the scale-up of the glucose dehydration to HMF, which holds the potential to qualify for the feedstock portfolio of our member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.3 -** Deoxygenation of Fatty Acids

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Robert Davis	**02/06/13**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Robert J. Davis, University of Virginia

Faculty: James Dumesic, University of Wisconsin-Madison; Matthew Neurock, University of Virginia; George A. Kraus, Iowa State University

Graduate Students and Staff: Juan A. Lopez-Ruiz, University of Virginia; Sikander Hakim, University of Wisconsin-Madison;

Statement of Project Goals

The overall goal of this work is the selective catalytic production of alpha-olefins by the decarboxylation of biomass derived lactones and carboxylic acids and to elucidate the factors that control selectivity in these reactions.

Project's Role in Center's Strategic Plan

One of the integrative test beds in this Center involves the production of alkenes. Since fatty acids are readily produced by fermentation (Thrust 2), an efficient catalyst that converts fatty acids into alpha-olefins (plus CO and water) is needed. Alpha-olefins have established utility in a variety of applications such as co-monomers for the production of low-density polyethylene (LDPE), polypropylene and other polymeric materials; as precursors to detergents, synthetic oils and plasticizers; and in the production of many specialty chemicals. The demand for α-olefins is growing and there is an imperative need to produce these high value compounds from non-petroleum based feedstocks. This project focuses on the use of heterogeneous catalysts for the selective conversion of biomass-derived lactones and carboxylic acids to α-olefins. This work is relevant to the Center's strategic interests in the selective production of value added terminal olefins from renewable resources feedstocks.

Fundamental Barriers and Methodologies

The transformation of fatty acids into α-olefins has been studied very little compared to the conversion of fatty acids to saturated hydrocarbons. A major fraction of previous work in the area of deoxygenation of carboxylic acids uses palladium as the transition metal catalyst and also dihydrogen, H_2, to prevent the catalyst from rapidly deactivating. Unfortunately, α-olefins are readily hydrogenated into paraffins in the presence of H_2 and transition metal catalysts. Thus, the work in this project explores catalyst compositions and reaction conditions that allow for decarbonylation while minimizing the subsequent conversion of product olefins. An important undesirable side reaction is water gas shift, WGS, since it produces hydrogen that could saturate the product olefins. We found that when catalyst supports that were effective at ketonization were tested, the catalysts were less effective than those

prepared with inert carbon supports. Thus, we concluded that ketonization is not an important reaction in the overall route to olefins but is instead a side reaction that consumes the fatty acid.

A new methodology involving a solid-acid catalyzed decarboxylation reaction to produce α-olefins has been added to this project. We demonstrated the catalytic process for *gamma* valerolactone (GVL) to selectively produce 1-butene. In our previous studies, we observed that the presence of either a C=C or a lactone is necessary for appreciable rates of decarboxylation to occur. Our initial studies with a SiO_2-Al_2O_3 catalyst (possessing both Brönsted and Lewis acid sites) revealed that although the catalyst offers high decarboxylation activity for GVL or its derivative pentenoic acids (PEA), it can also catalyze double bond isomerization in butene. In order to maximize the yield of 1-butene over SiO_2-Al_2O_3 catalysts, it was found to be necessary to remove 1-butene by flowing an inert sweep gas through the reactor, or to work at low butene yields. In our recent work, we observed that by using a solid acid catalyst consisting primarily of Lewis acid sites such as γ-Al_2O_3, it was possible to selectivity produce 1-butene at higher total butane yields as compared to SiO_2-Al_2O_3 catalyst and without the need to flow sweep gas through the reactor.

Achievements

Metal-Catalyzed Decarbonylation

We have successfully identified a catalyst and reaction conditions that produces α-olefins from carboxylic acids without the addition of small amounts of dihydrogen to maintain activity. More specifically, we have tested the liquid-phase and gas-phase conversion of heptanoic acid to 1-hexene on supported platinum on activated mesoporous Norit carbon. Liquid-phase reaction experiments were more selective towards the formation of isomers of 1-hexene, because of the rapid metal-catalyzed isomerization. High conversion experiments were more selective towards the formation of hexane. Furthermore, in liquid-phase reaction, the catalyst was very stable for a period of 120 hours at 573 K and 37 bar. The experiments performed on Pt/Norit C suggest that the rate of the decarbonylation reaction was not influenced by mass transfer limitation. Table 1 compares the performance of Pt/Norit C reduced at various temperatures whereas Table 2 compares catalysts with different Pt loading.

Table 1. Effect of metal dispersion on the decarboxylation/decarbonylation of heptanoic acid over 3 wt% Pt/Norit C. The reactions conditions were 573 K, 37 bar, 0.01 cm^3min^{-1} of feed composed by 95 wt% heptanoic acid and 5 wt% dodecane. These results were obtained after 20 hours of reaction, and conversion was corrected for background conversion on C.

Reduction Temperature (K)	Dispersion [a] (%)	TOF [b] (s^{-1})	Conversion (%)	Product Selectivity (%)					
				Liquid					Gas
				α-Olefin	i-Olefins	Paraffin	Ketone	Others	CO
623	49.0	0.0052	9.1	5.2	63.2	27.7	1.2	2.7	57.7
673	21.3	0.0051	3.8	5.0	62.9	28.2	1.4	2.5	69.1
773	12.7	0.0052	2.4	4.8	61.1	31.5	0.5	1.6	66.6
873	13.4	0.0051	2.5	5.0	54.7	34.0	4.0	2.3	55.4

[a] Result obtained by H_2 chemisorption
[b] Result calculated using the dispersion obtained by H_2 chemisorption

Table 2. Results of the Koros-Nowak criterion performed on 0.25 g of supported Pt on Norit carbon catalysts. The catalyst were tested at 37 bar and 0.01 cm^3min^{-1} of feed composed by 95 wt% heptanoic acid and 5 wt% dodecane. These results were obtained after 20 hours of reaction, and conversion was corrected for background conversion on C.

Loading (wt % Pt/C)	Dispersion [a] (%)	Pt Particle Size (nm)		Conversion (%)		TOF [c] (s^{-1})	
		Chemisorption [a]	TEM [b]	553 K	573K	553 K	573 K
1.0	28.2	4.0	1.8 ± 0.4	0.7	1.7	0.0022	0.0052
3.0	49.0	2.3	1.9 ± 0.2	-	9.1	-	0.0052
5.0	30.1	3.7	1.9 ± 0.3	4.4	8.2	0.0024	0.0045
10.0	39.0	2.9	2.1 ± 0.5	10.1	24.3	0.0021	0.0051

[a] Result obtained by H_2 chemisorption
[b] Result obtained by TEM analysis
[c] Result calculated using the dispersion obtained by H_2 chemisorption

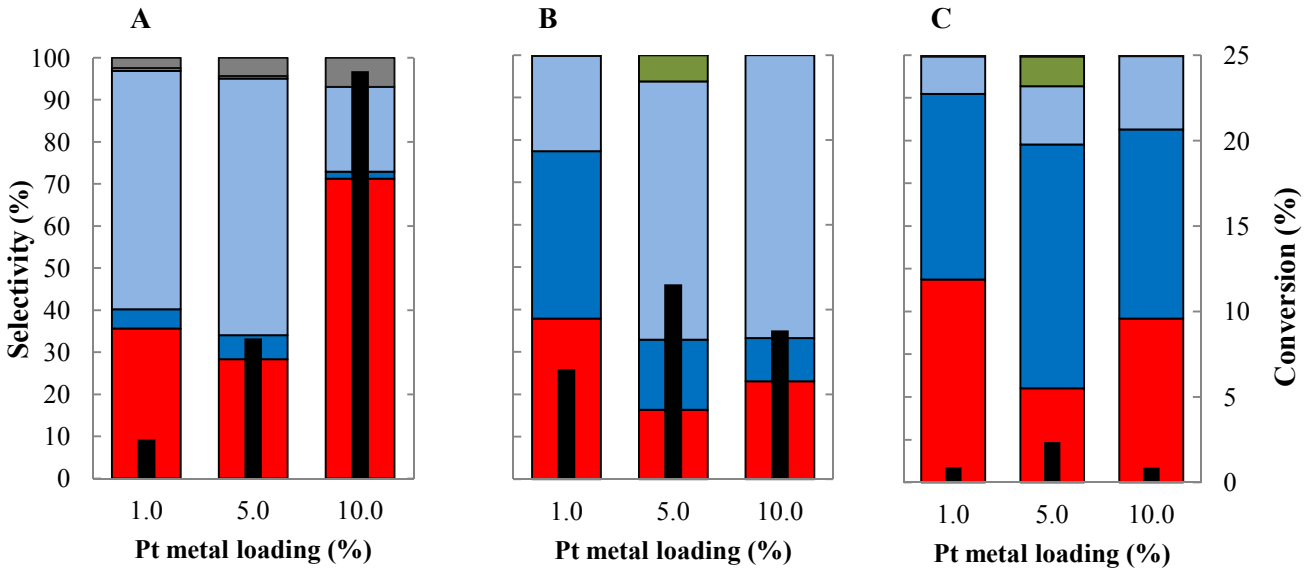


Figure 1. Effect of reaction pressure and feed flow rate on catalyst activity and product distribution over Pt/Norit carbon with different loadings of Pt at 573 K. The color bars can be read on the left axis and represents the product distribution. The black bars represent the conversion and can be read on the right axis. represents hexane, represents 1-hexene, represents i-hexenes, represents 7-tridecanone, and represents the unknown products. Figure A was run in liquid phase (37 bar) conditions and flow rate of 0.01 cm^3min^{-1}. Figure B was run in gas phase (1 bar) conditions and a flow rate of 0. 01 cm^3min^{-1}. Figure C was run in gas phase (1 bar) conditions and a flow rate of 0.05 cm^3min^{-1}. These results were obtained after 20 h of reaction.

Metal loading experiments, as summarized in Figure 1, showed that there is a shift in product selectivity with conversion as previously shown in the literature [7,20]. However, both olefins and CO are seen in high selectivity at low conversion, both in liquid and gas phase reaction experiments, which revealed that the main reaction path is decarbonylation under our reaction conditions (high heptanoic acid concentrations, no H_2 co-fed, 573 K, and 37 to 1 bar). The CO_2 formation and olefin hydrogenation to paraffin were likely the result of water gas shift ($CO + H_2O = CO_2 + H_2$) occurring rapidly under these conditions. Therefore, decarboxylation products such as CO_2 and hexane are attributed to side reactions, as illustrated in Figure 2. Furthermore, internal olefins are easily formed from the α-olefin by isomerization.

In summary, supported metal catalysts are being modified and characterized to understand the effects of composition, metal loading, particle size, and preparation conditions on the TOF, product selectivity, and catalyst stability in fatty acid decarbonylation.

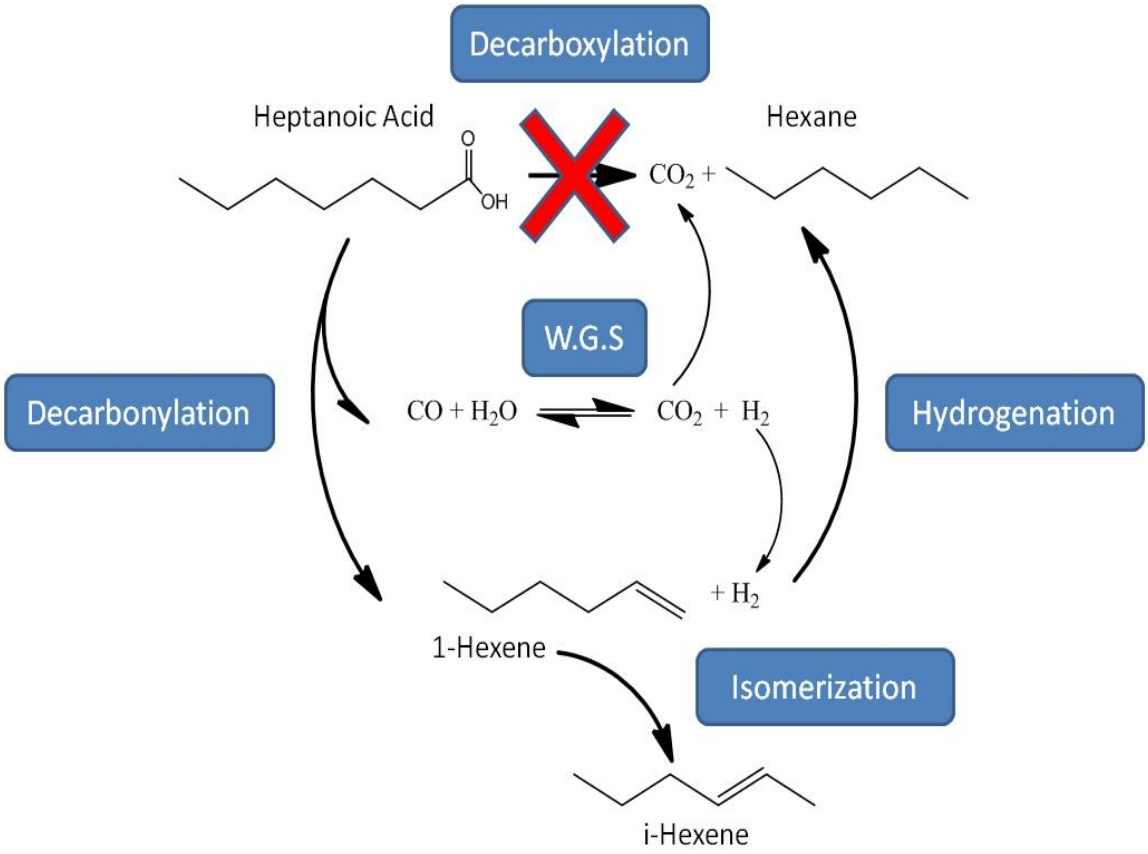


Figure 2. Working model for the decarbonylation/decarboxylation of heptanoic acid over supported Pt catalyst.

Acid-Catalyzed Decarboxylation

The major finding of our work is that a solid acid catalyst that contains primarily Lewis acid sites, is efficient in selectively producing 1-butene from the decarboxylation of γ-valerolactone (GVL). The results presented here are for decarboxylation reactions carried out by using γ-Al_2O_3 as a catalyst. Figure 3 summarizes the product distribution from the reaction of GVL and 2-pentenoic acid (2PEA). The reactions were performed in a fixed bed reactor loaded with 1 g of the catalyst. Feed was pumped in using a syringe pump at varying flow rates to achieve the desired weight hourly space velocity (WHSV), h^{-1}. It was observed that the inter-conversion between GVL and PEA is reversible and occurs rapidly relative to the rate of decarboxylation. The results indicated that γ-Al_2O_3 is not only active for decarboxylation but most importantly allows to achieve a percentage of 1-butene as high as ~99% in the final products. At 648 K and a space velocity of 1.8 h^{-1}, a 12% total butene yield was achieved; 98.5% of which was 1-butene (Figure 3). With a lower space velocity of 0.18 h^{-1}, higher butene yield of 49% could be achieved; 93% of which constituted 1-butene. Reactions at higher temperature of 723K resulted in even higher yields of butene at 61%, with 1-butene constituting 82% of total butene produced.

Feed (30% in H_2O)	T (°C)	WHSV (h^{-1})	GVL Conv. (%)	Butene Sel. (%)	1-Butene (%)	C5 Products* Sel. (%)	Acetic Acid Sel. (%)	Others Sel.** (%)
GVL	375	1.8	62	19	98.5	46	12	13
GVL	375	0.18	97	51	93.2	3	17	19
GVL	450	1.8	99	62	82.4	2	8	19
2PEA	375	1.8	88	17	98.3	54	9	9
2PEA	375	0.5	92	32	96.3	33	10	12

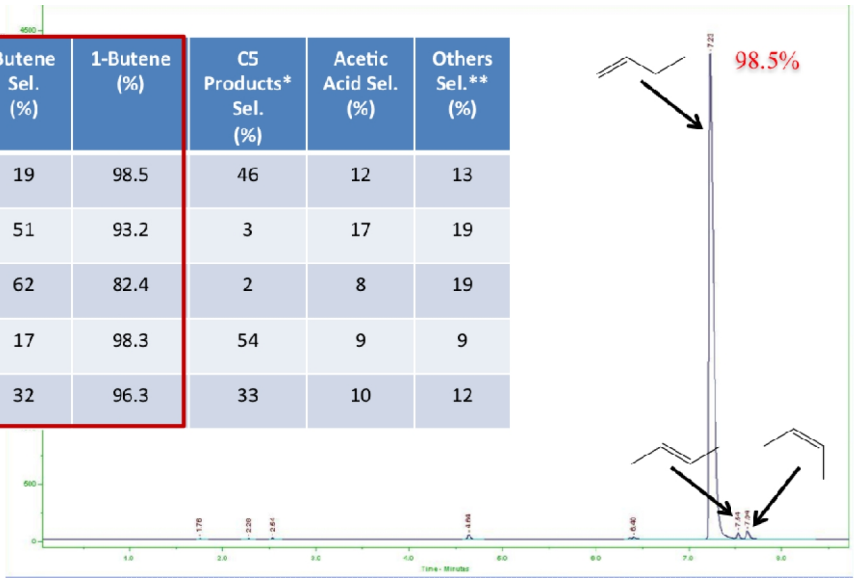


Figure 3. Product distribution from the reaction of γ-valerolactone (GVL) and 2-Pentenoic acid (2PEA) over γ-Al_2O_3 catalyst; *C5 products from GVL are 2-, 3- and 4-PEA, whereas from 2PEA are GVL, 3- and 4- PEA, ** Others include light hydrocarbon and condensation products of unsaturated acids and butene.

Similar results were obtained with 2-pentenoic acid as a feed, where a butene yield of 15% (of which 98% was 1-butene), and a butene yield of 30% (of which 96% was 1-butene) was achieved at a space velocity of 1.8 h^{-1} and 0.5 h^{-1} respectively. The main side products included equimolar amounts of acetic acid and propanal, which we believe are produced by the reaction of a PEA molecule with water (Figure 4). Other side products included condensation products between PEA and butene and light hydrocarbons.

The work here demonstrates the efficient use of an economical solid acid catalyst for the selective production of 1-butene from γ-valerolactone (GVL). The work presented here for GVL can be extended to other biologically derived lactones and unsaturated carboxylic acids to obtain corresponding value added α-olefins. A fundamental understanding of the competitive adsorption of species involved in the reaction network as well as the reactions taking place on the catalyst sites of this class of solid acid catalysts will help us to understand the factors controlling the selectivity and would allow for rational catalyst design.

Plans for the Next Five Years

Metal-Catalyzed Decarbonylation

We are working to optimize the catalyst preparation conditions to improve reaction rate and selectivity towards the formation of alpha-olefins. We will study the effect of bimetallic catalysts on the overall catalyst performance and also we will perform mechanistic studies to unravel the key reaction steps that lead to the formation of alpha-olefins from carboxylic acids. Furthermore, catalyst regeneration studies will be performed to understand the deactivation mechanism.

Acid-Catalyzed Decarboxylation

Next plan of action includes two major areas of focus: (i) detailed kinetic study will be performed in order to elucidate the reaction mechanism for GVL decarboxylation. Based on the previous work from our group and our

current experimental results we have proposed a reaction scheme illustrated in figure 4; space velocity studies with GVL and PEA isomers will provide useful insight. Additionally, isomerization reaction of butene over the same catalytic sites will be explored and the effect of water will be examined; (ii) other catalysts will be examined for elucidating the role of acid functionality in the selective production of 1-butene. While our preliminary results indicates that weaker Lewis sites are desirable for the selective production of 1-butene, in order to understand the role of Lewis acidity we will explore other Lewis acids such as ZrO_2 and Sn-Beta zeolite with weaker and stronger Lewis acid sites, respectively. A comparative study with these and other catalysts will potentially enhance the understanding of the selectivity towards 1-butene as well as the formation of byproducts observed in the product distribution. Eventually, the study will be extended to other lactones and unsaturated carboxylic acids.

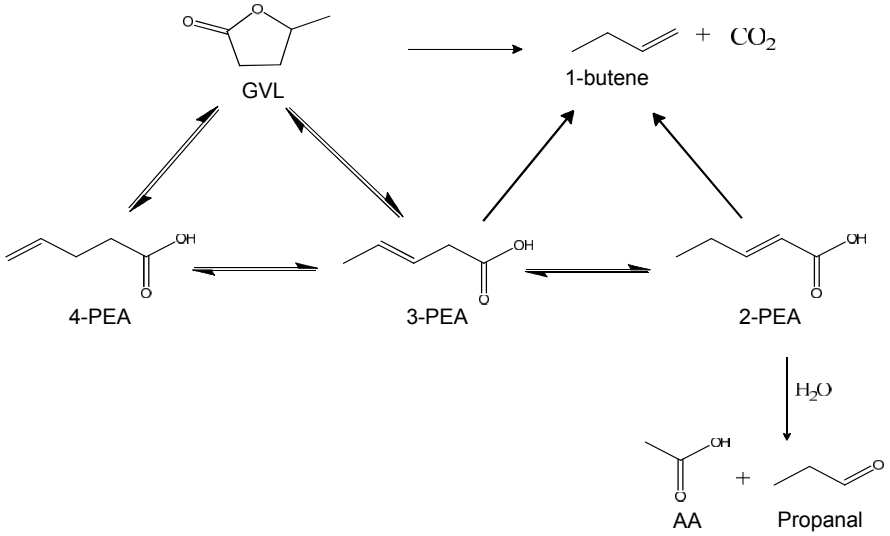


Figure 4: Proposed reaction network for GVL

Expected Milestones and Deliverables

The project will determine optimum rates and selectivities that can be used for process design calculations. A more detailed study with γ-Al_2O_3 will be performed in the next quarter to gain insight into the reaction mechanism. Other catalysts such as ZrO_2 and Sn-BEA will also be examined for their performance in the selective production of 1-butene in the next quarter.

Member Company Benefits

Members will have access to results from experimental studies of selective decarbonylation/decarboxylation reactions catalyzed by supported metal and acid catalysts. The member companies will also have access to catalyst synthesis and characterization techniques for supported metal and acid catalysts.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.5 -** Ring Opening Reactions

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
James Dumesic	**02/07/2013**	**03/01/2012 to 02/28/2013**

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Statement of Project Goals

The overall goal of this work is to develop catalysts for the selective ring-opening and hydrogenation of heterocyclic compounds derived from biomass and to elucidate the factors that control selectivity in these reactions.

Project's Role in Center's Strategic Plan

The ability to perform selective ring-opening of heterocyclics over heterogeneous catalysts is essential for the deoxygenation of biomass-derived feedstocks to produce value-added chemicals. In this project, we examine the hydrogenolysis of C-O bonds of furans and pyrans, ring-opening and decarboxylation of pyrones, and the factors that affect product selectivities in these reactions. This work is in line with the Center's strategic interests in selective ring-opening, hydrogenolysis, and upgrading of pyrones.

Fundamental Barriers and Methodologies

The main challenge for achieving selective ring-opening of furans and pyrans is to selectively cleave specific C-O bonds in these molecules in the presence of other similar bonds. We have focused our studies on understanding the nature of the active site on a rhodium-rhenium catalyst which facilitates highly selective ring-opening of tetrahydropyran-2-methanol and tetrahydrofurfuryl alcohol to their respective α,ω-diols. Previously, our experimental data and quantum chemical calculations suggest that this catalyst has both acid and metal sites (i.e., bifunctional). Our recent studies on this catalyst aim to provide more evidence for acidity and its correlation with C-O hydrogenolysis activity through the use of fructose dehydration as a probe reaction and correlating catalytic activity with acid site density changes with varying catalyst pretreatment temperatures. Our experimental work in the ring-opening of pyrones demonstrated that the ring-opening and decarboxylation of certain 2-pyrones proceeded in the absence of catalyst with water as the solvent. To better understand the role of the solvent and

chemistry behind ring-opening and decarboxylation, experiments employing an aprotic and protic solvent, probe molecules, and the presence or absence of an acid catalyst were used to establish reactivity trends. Apparent activation energy barriers were also measured for selected reactions. Mechanistic insight into ring-opening and decarboxylation were obtained through the use of quantum chemical calculations, and common reactivity rules for 2-pyrones established.

Achievements

To examine the effect of pretreatment temperature on the catalytic activity of 4 wt% $RhReO_x$/C (1:0.5), C-O hydrogenolysis activities were investigated under continuous flow reaction conditions. As shown in Figure 1, C-O hydrogenolysis rates of 2-hydroxymethyltetrahydropyran (HMTHP) decreased with increasing pretreatment temperatures. It was found that deactivation of the catalyst pretreated at 393 K was significant, while catalysts pretreated at 523 K and 723 K were fairly stable.

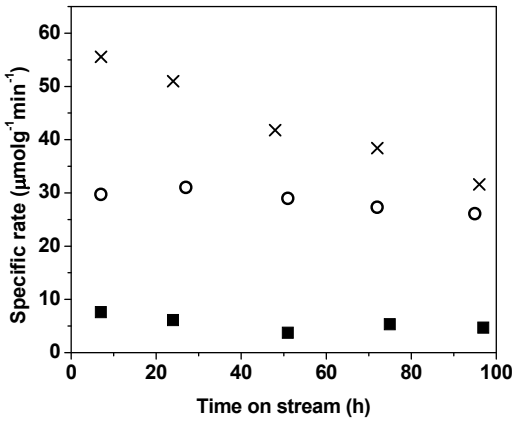

Figure 1 Hydrogenolysis of 2-hydroxymethyltetrahydropyran (HMTHP) over 4 wt% $RhReO_x$/C under continuous flow reaction conditions. Catalyst pretreated in flowing hydrogen at 393 K (×), 523 K (○) and 723 K (■) prior to initiation of liquid feed. Conversion levels of HMTHP at TOS_{8h} were 9%, 18% and 10% for 393 K (×), 523 K (○), and 723 K (■), respectively. Selectivities to 1,6-hexanediol were >90% for all data points. Reaction conditions were: 5 wt% 2-hydroxymethyltetrahydropyran in water as feed, $WHSV_{393K}$=4.2 h^{-1}, $WHSV_{523K}$=1.1 h^{-1}, $WHSV_{723K}$=0.5 h^{-1}, 393 K, 500 psi H_2.

To determine the changes in acid site density with pretreatment temperature, the NH_3 desorption profiles of 4 wt% $RhReO_x$/C (1:0.5) pretreated at various temperatures, and the monometallic catalysts 4 wt% Rh/C and 3.6 wt% Re/C, were obtained and are presented in Figure 2. Acid site densities for all catalysts were determined by integration of area under desorption peaks and the results shown in Table 1. The acid site density over 4 wt% $RhReO_x$/C (1:0.5) was found to decrease with increasing pretreatment temperatures. In our previous work, the number of metal sites over 4 wt% $RhReO_x$/C (1:0.5) were determined through measurement of the extent of irreversible CO uptake at 298 K for catalysts pretreated at the same temperatures employed here[1]. Based on data from both CO and NH_3 titrations, the molar ratio of metal to acid sites for 4 wt% $RhReO_x$/C (1:0.5) pretreated at 393 K, 523 K, and 723 K were determined to be 2.2, 4.3, and 16.9, respectively. Note that we corrected the acid site counts for 4 wt% $RhReO_x$/C (1:0.5) with that of the monometallic 4 wt% Rh/C catalyst to ensure that only acid sites for Re species are taken into consideration in these calculations; these values are denoted as "corrected acid site density" in Table 1.

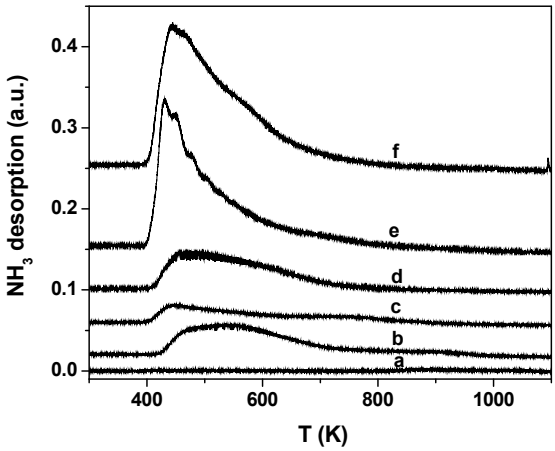

Figure 2 NH_3 temperature-programmed profiles for: (a) Vulcan carbon, (b) 4 wt% Rh/C pretreated in flowing H_2 at 523 K, (c) 3.6 wt% Re/C pretreated in flowing H_2 at 523 K, (d) 4 wt% RhRe/C (1:0.5) pretreated in flowing H_2 at 723 K, (e) 4 wt% RhRe/C (1:0.5) pretreated in flowing H_2 at 523 K, and (f) 4 wt% RhRe/C (1:0.5) pretreated in flowing H_2 at 393 K.

To obtain TOF values for C-O hydrogenolysis, we normalized the rates of HMTHP hydrogenolysis measured at 8 h time-on-stream (TOS) under continuous flow reaction conditions over 4 wt% $RhReO_x$/C (1:0.5) catalysts pretreated at various temperatures with the corrected acid site densities. C-O hydrogenolysis rates at 8 h TOS were used as these are the first steady-state data points obtained and most indicative of catalytic activity prior to the onset of appreciable deactivation. As shown in Table 1, TOF values for C-O hydrogenolysis of HMTHP were found to be similar for all pretreatment temperatures examined, indicating that catalytic activity appears to be correlated with the number of acid sites present over 4 wt% $RhReO_x$/C (1:0.5). The comparable TOF values obtained here are consistent with our previously proposed bifunctional C-O hydrogenolysis mechanism.

Table 1 Effect of pretreatment temperature on acid site density and catalytic activity for hydrogenolysis of 2-(hydroxymethyl)tetrahydropyran over 4 wt% RhRe/C (1:0.5) under continuous flow reaction conditions.[a]

Catalyst	Pretreatment T (K)	Total acid site density[b] ($\mu molg^{-1}$)	Corrected acid site density[c] ($\mu molg^{-1}$)	Conv (%)	Selectivity to 1,6-HDO (%)	Specific rate at 8h TOS ($\mu molg^{-1}min^{-1}$)	TOF[d] (min^{-1})
RhRe	393	95	65	9	90	56	0.86
RhRe	523	64	34	18	96	30	0.88
RhRe	723	38	8	10	95	7	0.93
Rh	523	30					
Re	523	17					

[a]Reaction conditions described in Figure 1. [b]Determined by NH_3 temperature-programmed desorption (Figure 2). [c]Corrected acid site density = (Total – Rh/C) acid site density. [d]TOF calculated using corrected acid site density.

To obtain further evidence of Brønsted acidity over 4 wt% $RhReO_x$/C (1:0.5), an acid-catalyzed probe reaction, namely fructose dehydration to 5-hydroxymethylfurfural (HMF), was used. Figure 3 shows results for the dehydration of fructose over 4 wt% $RhReO_x$/C (1:0.5) which was pretreated at 523 K in flowing hydrogen prior to initiation of liquid feed flow. A single-phase reaction solvent was employed, specifically a mixture of THF and water (mass ratio of THF: water = 4:1), as this solvent system has been previously shown to be effective

for fructose dehydration to HMF under continuous flow reaction conditions[2]. Interestingly, we found that 4 wt% $RhReO_x$/C (1:0.5) displayed substantial activity for HMF formation with selectivities to HMF maintained at approximately 50% at a conversion level of 30%. Significantly, the catalyst was fairly stable with TOS and specific formation rates of HMF were maintained at ~1.8 $\mu molg^{-1}min^{-1}$ for up to 250 h TOS. The formation of HMF from fructose here provides further evidence of the existence of Brønsted acidity over 4 wt% $RhReO_x$/C (1:0.5).

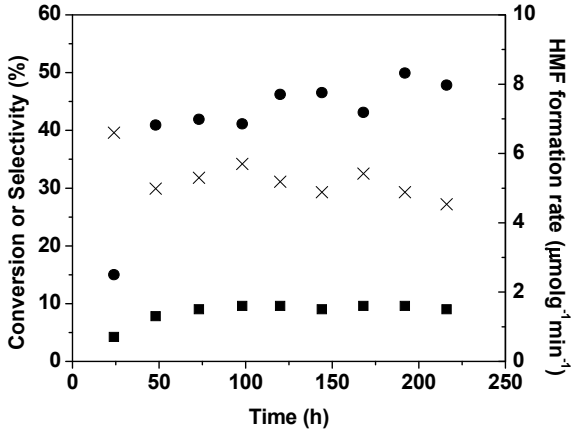

Figure 3 Conversion of fructose (×), selectivity to 5-hydroxymethylfurfural (•), and specific formation rate of HMF (■) as a function of time on stream over $RhReO_x$/C catalysts pretreated at 523 K. Reaction conditions: 300 psi He, 403 K, 2 wt% fructose in THF/water (mass ratio of THF: water = 4:1) as feed, WHSV =0.1h^{-1}.

In studies for 2-pyrone ring-opening and decarboxylation, we obtained reaction data using 4-hydroxy-6-methyl-2-pyrone (**1**, also known as triacetic acid lactone), 5,6-dihydro-4-hydroxy-6-methyl-2H-pyran-2-one (**3**), and 4-hydroxy-6-methyltetrahydro-2-pyrone (**6**) as reactants, tetrahydrofuran and water as solvents, with and without an acid catalyst (Amberlyst 70). The reactions studied are shown in Figure 4. It was found that while **1** and **3** ring-open and decarboxylate in water without the presence of catalyst, **6** was unreactive under similar conditions.

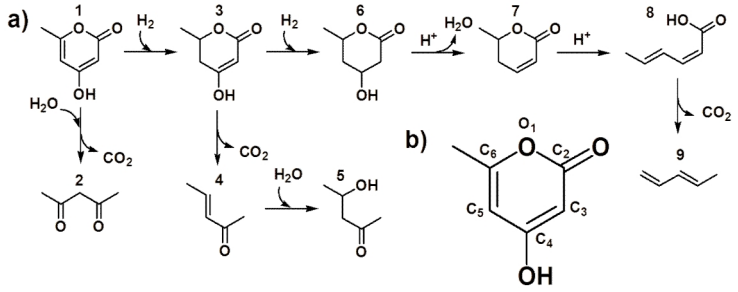

Figure 4. a) Compounds are as follows: 4-hydroxy-6-methyl-2-pyrone/ triacetic acid lactone (1); 2,4-pentanedione (2); 5,6-dihydro-4-hydroxy-6-methyl-2H-pyran-2-one (3); 3-penten-2-one (4); 4-hydroxy-pentanone (5); 4-hydroxy-6-methyltetrahydro-2-pyrone (6); parasorbic acid (7); sorbic acid (8); 1,3-pentadiene (9); b) Nomenclature for the ring-carbon and ring-oxygen atoms of 1.

Reaction mechanisms were proposed for the ring-opening and decarboxylation of **1** and **3**, involving initial keto-enol tautomerization steps (KET) followed by nucleophilic attack of water and retro-Diels Alder (rDA), respectively. The measured activation energy barriers for ring-opening and decarboxylation of **1** and **3** were found to agree well with calculated values

based on the proposed reaction mechanisms. A proposed ring-opening and decarboxylation mechanism for **3** is shown in Figure 5. We suggest that ring-opening of **3** proceeds through KET to **3a** followed by formation of the enolic isomer, 3, 6-dihydro-4-hydroxy-6-methyl-pyran-2-one (**3b**). These KET reactions occur rapidly due to their low DFT-predicted activation barriers of 34 (**3** to **3a**) and 22 kJ/mol (**3a** to **3b**), respectively (Figure 5b), compared to the higher barrier for decarboxylation (49 kJ/mol). The intermediate **3b** subsequently reacts through a retro-Diels-Alder (rDA) mechanism to produce 3-hydroxy-penta-1,3-diene (**3d**) and CO_2. Under conditions of full solvation (27 water molecules/unit cell), the rDA reaction was found to proceed in two steps through a zwitterion intermediate (**3b** to **3c**), followed by decarboxylation (**3c** to **3d**), with activation energy barriers of 31 and 49 kJ/mol, respectively. The **3d** diene that results from decarboxylation can undergo tautomerization (ΔE_a = 27 kJ/mol) to **4** (Figure 5a). The overall activation barrier for the ring-opening and decarboxylation of **3** is estimated to be 50 kJ/mol (Figure 5b), which agrees with the experimentally measured value of 42±18 kJ/mol (95% confidence interval) (Figure 5c).

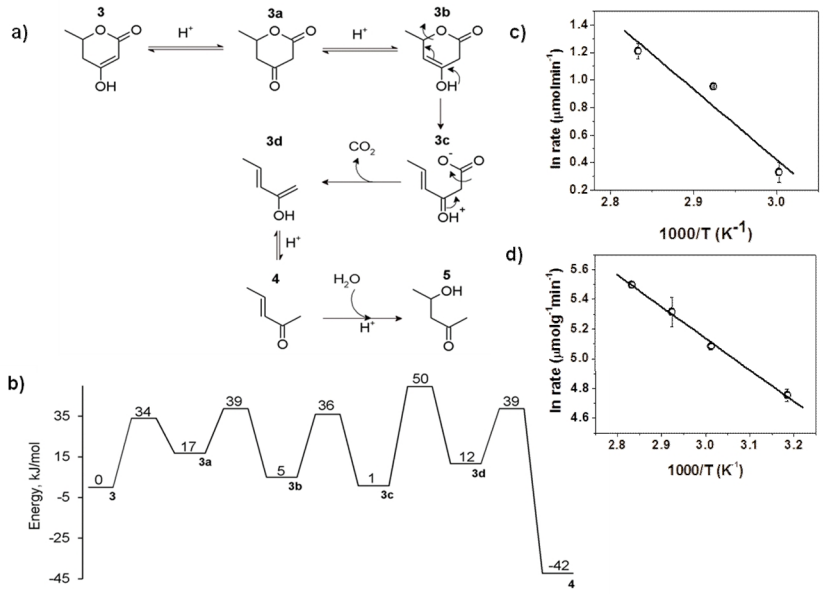

Figure 5. a) Proposed mechanism for the ring-opening and decarboxylation of 3 in water, b) DFT-calculated energy diagram for the reaction pathway of 3 to 4 in water, numbers indicate energy in kJ/mol, c) rates of thermally-activated ring-opening and decarboxylation of 3 at various reaction temperatures in water (no catalyst). 21 bar He, space time = 13 min. Measured apparent activation energy barrier = 42 ± 18 kJ/mol (95% confidence interval), d) rates of thermally-activated ring-opening and decarboxylation of 3 at various reaction temperatures in water over Amberlyst™ 70. 21 bar He, WHSV = 15 h^{-1}. Measured apparent activation energy barrier = 18 ± 4 kJ/mol (95% confidence interval).

Based on the ring-opening and decarboxylation mechanisms we have proposed for **3** and **6**, respectively, it is suggested that analogous structures with the C_4=C_5 bond in the ring should undergo decarboxylation in the absence of a catalyst via the rDA reaction. To probe this hypothesis, we carried out studies using probe molecules such as 3,6-dihydro-4,6,6-trimethyl-2H-pyran-2-one and isoparasorbic acid. Both probe molecules were observed to ring-open and decarboxylate in the presence of water, consistent with our proposed mechanisms.

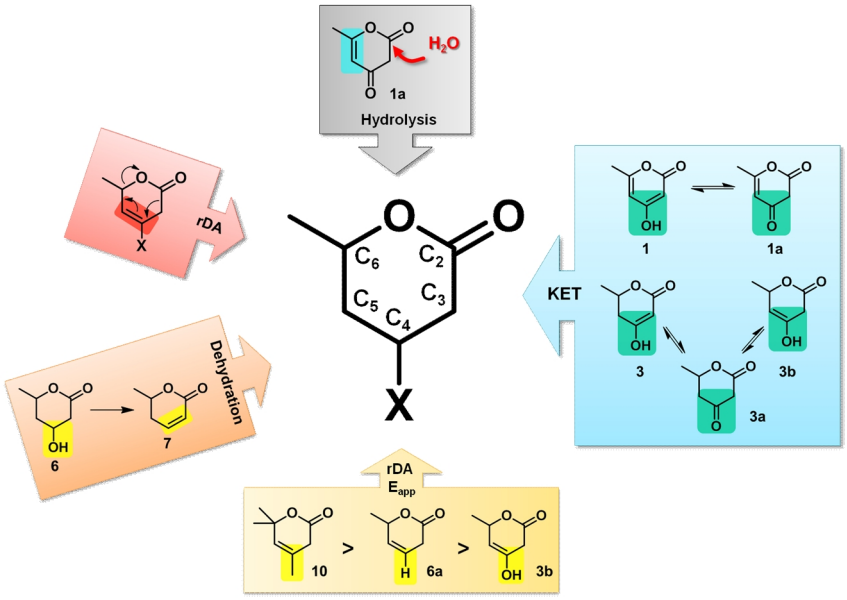

Figure 6. Overview of structure-reaction relationships for 2-pyrones. Abbreviations: retro-Diels Alder (rDA), keto-enol tautomerization (KET), and apparent activation energy barrier (E_{app}).

The reactivity trends we have observed for 2-pyrone molecules can be related to key structural features of this family of molecules. An overview of the structure-reaction relationships is displayed in Figure 6 and provides a universal set of rules by which 2-pyrones react. The observed ring-opening and decarboxylation of **3, 6** and **10** in water without the aid of a catalyst indicates that the presence of a C_4=C_5 bond in the ring is required to carry out the low energy rDA reaction and eliminate the CO_2 dienophile from the resulting diene. The nature of the substituent attached to the C_4 atom during the ring opening/decarboxylation reaction dictates whether the rDA reaction proceeds through a single concerted or a two-step mechanism through a stable zwitterion intermediate. Additionally, the polarized transition state and zwitterion intermediate in the rDA are stabilized by hydrogen-bonding in protic solvents like water. The overall rDA reactivity of the pyrone therefore depends on the electron withdrawing ability of the substituent attached to the C_4 site.

Although **1**, **3** and **6** differ by only one degree of unsaturation from one another, they undergo ring-opening and/or decarboxylation through different mechanisms. The presence of the C=C bonds adjacent to the C_4 hydroxyl group in the ring allows for the KET reactions observed for **1** and **3.** For **1**, the keto form is more reactive for the nucleophilic addition of water, which results in the formation of the β-keto acid, while the enol at the C_4=C_5 position of **3b** is responsible for the rDA reaction. Because **6** does not possess unsaturated C=C bonds within the ring, the C_4 hydroxyl group is unable to convert into a β-keto group and **6** does not undergo decarboxylation. Species **6**, however, can dehydrate in the presence of water and under acidic conditions to form **7.**

Other Relevant Work

The work here, in combination with efforts in selective dehydration by other projects in this Thrust, demonstrate the feasibility of obtaining commercially valuable α,ω-diols from biorenewable molecules (e.g., 1,6-hexanediol from HMF). Recent literature suggests that Pt, Ru, and Ir catalysts promoted with Re display high activity and selectivity in the hydrogenolysis of tetrahydrofurfuryl

alcohol[3], HMTHP[4], and glycerol[5-8] to their corresponding α,ω-diols. Results from this study provide the first consistent account for the nature of the active site in metal catalysts promoted with oxophilic additives for hydrogenolysis reactions, and provide guidance for the use of this new class of heterogeneous catalysts for the selective deoxygenation of biomass to fuels and chemicals.

Plans for the Next Five Years

Detailed characterization of the Rh-ReO_x/C catalyst will be performed, with emphasis on the use of in situ EXAFS to determine the oxidation state of Re, the coordination of Re with Rh and O, and how these catalyst properties vary under reaction conditions.

Expected Milestones and Deliverables

EXAFS data collected recently will be analyzed, interpreted, and published in the next quarter. The work on 2-pyrone ring-opening and decarboxylation chemistry is currently under review and will be published in the next quarter.

Member Company Benefits

Members will have access to unpublished results from experimental studies of the selective ring-opening reactions by supported metal catalysts, and ring-opening and decarboxylation mechanisms for 2-pyrones.

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NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.7 -** Hydrothermally Stable Catalysts and Catalyst Supports

Thrust: Research Thrust 3-Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Abhaya K. Datye	**02/14/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Abhaya K. Datye, University of New Mexico; Other Faculty: James A. Dumesic, University of Wisconsin-Madison; Brent H. Shanks, Klaus Schmidt-Rohr and Jean Phillipe Tessonier, Iowa State University

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Graduate Students: Jason Anderson, Robert L. Johnson and Alex Liu, Iowa State University

Postdoc: Haifeng Xiong, University of New Mexico

Research Assistant Professor: Hien N. Pham, University of New Mexico

Statement of Project Goals

The objective of this project is to develop catalysts and catalyst supports with improved hydrothermal stability in aqueous-phase reactions for biorenewable conversion processes.

Project's Role in Center's Strategic Plan

A central challenge for the production of biorenewable chemicals and fuels is the development of catalysts and supports that are hydrothermally stable during aqueous-phase reactions. Conventional catalysts and supports designed for gas-phase reactions may not be suitable for such reactions, particularly aqueous-phase reactions at temperatures in excess of 473 K, due to loss of surface area, aggregation of the support and sintering or leaching of the metal phase. Hence, part of the catalyst tool chest for biorenewable processing involves the development of stable catalysts and supports that can operate under aqueous conditions, with high activity and stability.

Fundamental Barriers and Methodologies

Mesoporous oxides, such as silica or alumina, are not hydrothermally stable at elevated temperatures due to grain growth, phase transformations and sintering resulting in loss of surface area. Likewise, solid acid catalysts, such as niobia, show loss of catalytic activity and stability in aqueous-phase reactions due to the transformation from amorphous to crystalline niobia, also resulting in loss of surface area. In previous work we showed that coating the pore walls of mesoporous silica SBA-15 support with thin carbon films can significantly improve its hydrothermal stability. In this report, we extend this approach to other commercially available oxides. High surface area carbon is stable under aqueous phase reactions, but lacks adequate

functional groups. Therefore we have developed a one pot hydrothermal synthesis that generates niobia nanoparticles embedded in carbon spheres which avoids the need to first functionalize the carbon surface. The niobia embedded in carbon provides excellent stability for aqueous-phase butanol dehydration. In the studies of carbon catalysts, NMR was used to study the sulfur-carbon bonds for model sulfonic acid compounds, and the results have given us insight into developing solid acid carbon materials with high catalytic activity and hydrothermal stability.

Achievements

Coating of Oxides with Carbon

An aqueous solution of sucrose was added to commercial silica or alumina (10 wt% carbon), and the mixture was stirred at room temperature until water was evaporated. The dried powder was incompletely pyrolyzed at 400°C for 2 h under UHP N_2 flow. For silica and carbon-silica, 0.5 wt% of Pd was deposited by room temperature alcohol reduction approach. The samples were subjected to treatments in liquid water in an autoclave vessel heated to 200°C at autogeneous pressure (22 bar) and held at this temperature for 12 h. Scanning transmission electron microscopy (STEM) images of the alumina samples (Figure 1) show that after hydrothermal treatment uncoated alumina loses 70% of its surface area due to a phase transformation from γ-Al_2O_3 to boehmite (γ-AlOOH), as determined by X-ray diffraction (XRD). In contrast, hydrothermal stability of alumina is significantly improved after carbon coating with no surface area loss, and retains its γ-Al_2O_3 phase after hydrothermal treatment. For 0.5 wt% Pd supported on uncoated and carbon-coated silica, STEM images of the silica samples (Figure 2) show that the as-prepared samples consist of highly dispersed, 1-1.5 nm sized Pd nanoparticles. After hydrothermal treatment, most of the Pd has been lost from the surface of uncoated silica. The remaining Pd on silica (< 0.1 wt% Pd) has sintered to form 2-20 nm sized particles, and this Pd sintering is accompanied by the grain growth and sintering of the uncoated silica support. In contrast, there is little to no leaching of Pd from carbon-silica and no change in the morphology of the support after hydrothermal treatment. Pd has sintered to form 2-5 nm sized particles, but the extent of sintering is significantly less than Pd on uncoated silica. Hence, Pd is significantly more stable when supported on hydrothermally stable carbon-coated-silica.

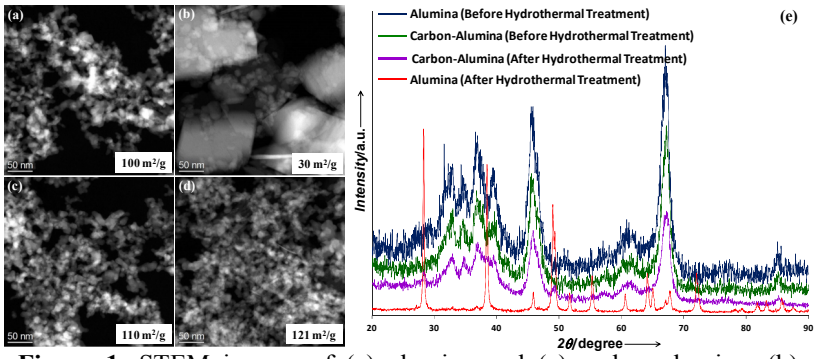


Figure 1. STEM images of (a) alumina and (c) carbon-alumina; (b) alumina and (d) carbon-alumina after treatment in liquid water at 200°C for 12 h. (e) XRD patterns of uncoated and carbon-coated alumina samples, before and after hydrothermal treatment.

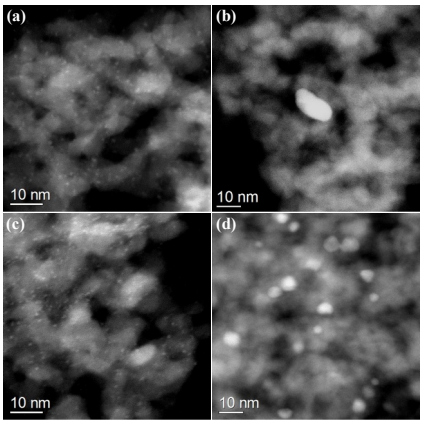


Figure 2. STEM images of 0.5 wt% Pd supported on (a) silica and (c) carbon-silica; (b) 0.5 wt% Pd/silica and (d) 0.5 wt% Pd/carbon-silica after hydrothermal treatment.

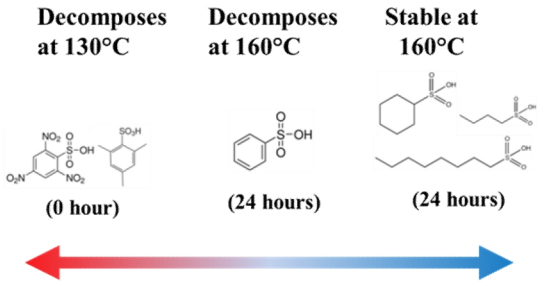
Chemical Structure of Sulfonated Carbon Materials from NMR

A suite of carbon and sulfonated-carbon materials were synthesized in a range of temperatures and conditions to test the effect of functional groups and carbon backbones on the hydrothermal stability of the sulfonate active sites. These catalysts matched catalysts from current research according to typical characterization. We were able to successfully model average chemical structures of these materials on the order of several hundred atoms using advanced two-dimensional NMR experiments and quantitative 1-D ^{13}C NMR spectra. These results show significant differences in the spectra of materials synthesized over a wide range of temperatures - a conclusion not obtained by typical characterization methods. However, we found all of these materials to have poor stability when subjected to hydrothermal conditions. This indicates that the carbon backbone does not have as strong an influence as presupposed by literature on the stability of the acid sites.

Model Sulfonic Acid Compounds: Probing the Relative Sulfur-Carbon Bond Strength

In order to provide a bond-level understanding of our previous hydrothermal stability data, we investigated the carbon-sulfur bond. In this work, we chose several molecules for stability analysis representing the immediate chemical nature of the active sulfonic acid group on a carbon backbone: aromatic, saturated cyclics, and straight-chain aliphatic. This work sought to investigate a literature hypothesis postulating that an aromatic backbone would be better than an aliphatic one and the electron withdrawing nature of the many functional groups on the carbon would lead to its stability. This led to the use of trimethylbenzene sulfonic acid, trinitrosulfonic acid, and benzenesulfonic acid, exemplifying electron donation, withdrawal and a control, respectfully. We compared alkyl-cyclic versus aromatic benzene sulfonic acid versus cyclohexane sulfonic acid. We explored the chain length dependence with methane sulfonic acid, butane sulfonic acid, and octane sulfonic acid. We placed each sulfonic acid into a pressurized reactor at 100-160°C and time dependent samples were taken. We used solution NMR for species identification. The results follow in the figure below. Nothing happened to any of the model compounds at 100°C. The trisubstituted benzenesulfonic acids showed the most susceptibility to hydrolysis: signals disappeared in the NMR spectra at 130°C already at time zero, i.e. during the heat up time of the reactor. The unsubstituted benzenesulfonic acid was hydrolyzed only after 24 h of 160°C water. The aliphatic compounds remained unperturbed throughout the entire process and withstood the 24 h in 160°C water. This strongly suggests that the current electrophilic aromatic substitution method generates aromatic sulfonic acid active sites that are less stable than aliphatic sulfonic acid groups.

Development of Hydrothermally Stable Solid Acid Catalyst Materials (Aqualent)



Results from our testing of model compound experiments show that alkyl sulfonate bonds show little degradation compared to aromatic sulfonates when subjected to hydrothermal treatments. From these results we sought to create a carbon material where sulfonate group are attached through an alkyl linker to the aromatic backbone. We were able to successfully accomplish this goal, and achieved 3-4x's higher sulfur content than with previous methods, and the new materials show high catalytic activity and hydrothermal stability. Additionally these materials have been grafted onto a macroporous silica template, inspired by our successful results on the protective nature of a carbon layer on a silica surface.

Niobia Nanoparticles Embedded in Carbon Spheres (CS)

The embedded niobia/carbon was tested for aqueous-phase butanol dehydration to butene at 240°C and 51 bar (Figure 3), commercial HY-340 niobia deactivates quickly with time-on-stream (TOS) due to the transformation from amorphous niobia to large, crystalline niobia particles, as shown by HRTEM (Figure 4). In contrast, both 5Nb/CS-HT and 10 Nb/CS-HT (5Nb and 10Nb denote the nominal loadings of 5 and 10 wt% niobia, respectively) are very stable with TOS and their activity is higher than HY-340 niobia. No change was observed in the size of the niobia particles for 5Nb/CS-HT (as shown by STEM in Figure 4) and 10Nb/CS-HT after butanol dehydration. Thus, the high stability is due to the strong interaction between niobia and carbon. However, 20Nb/CS-HT deactivates with TOS due to the formation of large crystalline niobia particles, as confirmed by STEM, indicating that the niobia loading is too high for carbon to effectively interact with the niobia particles.

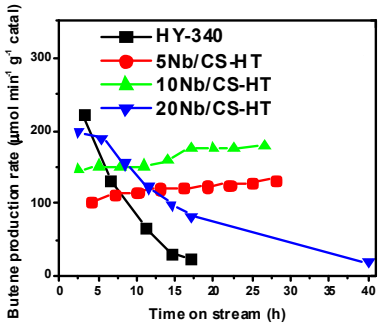


Figure 3. Production rate of butene as a function of time-on-stream from aqueous-phase butanol dehydration (reaction conditions: 240°C, 51 bar).

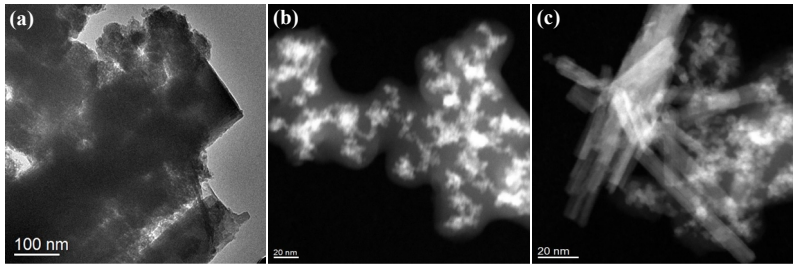


Figure 4. HRTEM image of (a) commercial HY-340 niobia after butanol dehydration. STEM images of (b) 5Nb/CT-HT and (c) 20Nb/CT-HT after butanol dehydration.

Other Relevant Work

Carsten Sievers at Georgia Tech is studying the hydrothermal stability of supports, and there is increasing interest worldwide as evident from publications in this area.

Plans for the Next Year

We plan to prepare Pd at different loadings (e.g., 5 wt%) deposited on oxide and carbon-coated oxide supports, and the resulting catalysts will be tested under hydrothermal conditions and compared to Pd deposited on bulk carbons. Also, we will study the hydrothermal stability of Pd on oxides that are coated with carbon, thereby forming a carbon overlayer around Pd nanoparticles. In parallel, we will study the catalytic properties of Pd both on carbon-coated oxides and on niobia/

carbons in several aqueous-phase reactions. We plan to validate the hydrothermal stability of the alkyl linked sulfonated carbons as well as optimize the synthesis procedure for these novel materials. The materials will be tested for hydrothermal stability and reactivity, and characterized using the NMR techniques developed in this project. Jason Anderson will be spending the 2013 summer at the Fritz Haber Institute, which will allow him to characterize these materials with techniques that complement the NMR analysis. We plan to explore the synthesis of the sulfonated carbons on high surface area supports such as mesoporous silica to enhance the catalytic properties of these solid acids.

We have expanded our studies of carbon based supports and catalysts with the addition of a new faculty member at Iowa State. J.-P. Tessonnier joined Iowa State University and CBiRC as a junior faculty in June 2012. The Tessonnier group has expertise in the synthesis, chemical functionalization and characterization of carbon materials for catalysis. He plans is to design carbon-based basic catalysts, a new and unexplored class of materials with potential applications for the isomerization, dehydration and aldol condensation reactions. Different amines have been screened for the homogeneously catalyzed isomerization of glucose to fructose in order to identify the most promising basic actives sites. A fructose yield of 30% was achieved under optimized reaction conditions, a performance similar to that of Sn-Beta, the state of the art catalyst for this reaction. Future plans include the grafting of the organic moieties on carbon nanotubes using a technique developed previously to synthesize triethylamine-functionalized nanotubes and alkyl-grafted graphene sheets.

Expected Milestones and Deliverables

A number of publications covering the NMR technique development and application of this to characterization of sulfonated carbons are in the process of being submitted. An invention disclosure has been submitted on the alkyl linked sulfonated carbons and further data are being taken to turn the disclosure into a patent filing. In the upcoming year, we expect to have an optimized alkyl linked sulfonated carbon catalyst that will be a low cost solid acid catalyst, which is hydrothermally stable.

Published Paper: "Improved Hydrothermal Stability of Mesoporous Oxides for Reactions in the Aqueous Phase," Pham et al., *Angew. Chem. Intl. Ed.*, 51 (2012) p. 13163-13167.

Member Company Benefits

Hydrothermally stable catalysts and supports have many potential applications in the conversion of biorenewable feedstocks such as dehydration, esterification, and ring-opening reactions.

Commercialization / Technology Transfer

BASF in Iselin, NJ is interested in the carbon coating approach and is currently testing these materials. Invention disclosures have been submitted on the improved hydrothermal stability of oxides by carbon coating and via niobia/carbon composites.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.9 -** Pyrone Conversions

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
James Dumesic	**02/14/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: James Dumesic, University of Wisconsin-Madison

Faculty: Klaus Schmidt-Rohr, George Kraus Iowa State University

Graduate Students and Staff: Mei Chia, Thomas Schwartz, University of Wisconsin-Madison; Jennifer Lee, Robert Johnson, Iowa State University

Statement of Project Goals

The overall goal of this work is to identify reactions and catalysts which are useful in the upgrading of pyrones to produce commodity or fine chemicals. Catalyst development focuses on the imparting of resistance to biogenic impurities which are present in the cell culture media used to produce the pyrones. Fundamental spectroscopic and computational studies are also being performed to elucidate the nature of catalyst inhibition by biogenic impurities such as amino acids.

Project's Role in Center's Strategic Plan

The successful upgrading of biologically-derived pyrones is predicated on catalysts which are not significantly inhibited by the presence of small amounts of residual cell-culture impurities. In this project, we examine the effect of biogenic compounds, such as amino acids, on the catalytic activity of supported metal catalysts for the hydrogenation of pyrones. This information is then used in the design of catalysts which are immune to inhibition by these compunds. This work is in line with the Center's strategic interests in pyrones, hydrogenation, and biogenic impurities.

Fundamental Barriers and Methodologies

Our work in the upgrading of pyrones revealed that the presence of small amounts of biogenic compounds in the reaction mixture resulted in decreased catalytic activity over metal catalyst for the hydrogenation of pyrones. This inhibitive effect of biogenic compounds was systematically examined through the use of controlled additions of amino acids to the reaction mixture and observation of their effect on catalytic activity. The focus of subsequent work has been to develop a catalyst which is unaffected by such inhibition. Experiments have focused on application of bimetallic alloys and polymer overcoating as a means of adding amino acid resistance to the hydrogenation catalyst. Additionally, investigations using ^{13}C solid-state NMR have been performed to gain insight about the mechanisms of catalyst inhibition by amino acids such as methionine.

Achievements

As part of efforts in the development of the Pyrone Testbed, we have previously reported[1] reactions using triacetic acid lactone (TAL), also known as 4-hydroxy-6-methyl-2-pyrone, as a feedstock for the production of several commodity chemicals (Figure 1). The catalytic transformations studied include selective hydrogenation, decarboxylation, dehydration, and acid-catalyzed ring-opening.

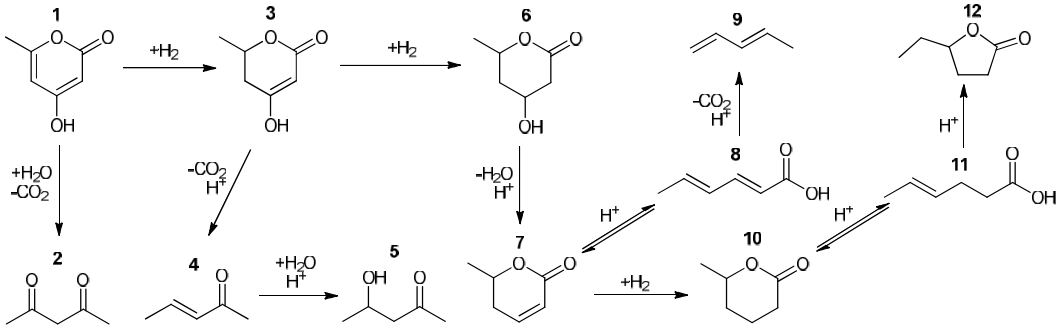


Figure 1. Reactions explored for the upgrading of pyrones to commodity chemicals. Compounds are as follows: 4-hydroxy-6-methyl-2-pyrone/ triacetic acid lactone (TAL); 2,4-pentanedione/ acetylacetone; 5,6-dihydro-4-hydroxy-6-methyl-2H-pyran-2-one; 3-penten-2-one; 4-hydroxy-2-pentanone; 4-hydroxy-6-methyltetrahydro-2-pyrone; 6-methyl-5,6-dihydro-2-pyrone/parasorbic acid; 2,4-hexadienoic acid/ sorbic acid; 1,3-pentadiene; δ-hexalactone; hexenoic acid; γ-caprolactone (adapted from ref. 1).

We noted that production of most value-added chemicals from TAL proceeds via hydrogenation of the unsaturated bonds in TAL and subsequent acid catalyzed steps, as shown in Figure 1. Owing to the fact that TAL readily decarboxylates in water, as described above, an adsorption scheme was used to recover TAL from spent fermentation media supernatant; however, traces of amino acids were also recovered. We observed that the presence of these compounds resulted in a complete loss of hydrogenation activity. Temperature-programmed reduction of a deactivated catalyst revealed the presence of amine (NH_x) fragments, as well as HS fragments, further suggesting that catalyst inhibition results from adsorption of amino acids. The action of amino acids on hydrogenating metals is not well documented in the literature, so we then undertook to study, qualitatively, the influence of these compounds by controlled addition to the reaction mixture. The degree to which TAL hydrogenation is inhibited is governed by the R-group of the amino acid. Six representative amino acids were investigated. Unsurprisingly, the sulfur-containing amino acids were the most inhibitory, while alanine was the least (containing only a methyl side chain). In order of decreasing effect, the amino acids studied were: Methionine (83% yield loss), Cysteine (77% yield loss), Tryptophan (71% yield loss), Phenylalanine (58% yield loss), Arginine (46% yield loss), and Alanine (25% yield loss).

Recent work has focused on development of a catalyst which is resistant to inhibition by amino acids. A two-pronged approach was taken in this regard. In the first prong, polymeric overcoating of the metal catalyst (see Figure 2) was used to provide a solid solvent permanently fixed above the metal site. The environment provided by this solvent was chosen such that that it would be favorable for adsorption of TAL, but unfavorable for adsorption of highly polar amino acids. We observed that TAL is soluble in moderately polar solvents (short chain alcohols such as ethanol and 1-butanol are best), while amino acids are soluble

only in highly polar solvents. Consequently, poly(vinyl alcohol) (PVA) has been chosen as an appropriate solid solvent which promotes adsorption of TAL while making adsorption of amino acids unfavorable.

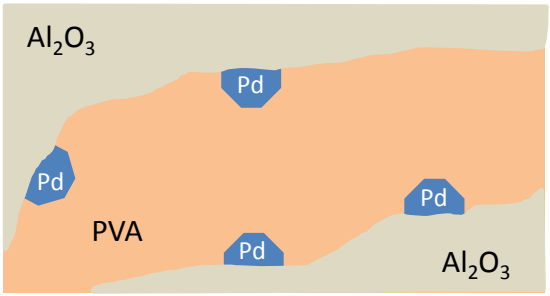


Figure 2. Schematic representation of alumina-supported palladium hydrogenation catalyst. The pores are filled with poly(vinyl alcohol) to improve tolerance towards amino acids.

Due to the significant inhibition observed for reactions carried out in the presence of methionine, we have chosen this as a representative case for inhibition by amino acids in general. As shown in Figure 3, the use of a PVA overcoating significantly improves the amino acid tolerance Pd/Al_2O_3. While the turnover frequency (TOF) is lower in the absence of methionine, as methionine is added to the reactor it becomes apparent that the PVA overcoated catalyst maintains much more of its initial activity than does the base case palladium catalyst.

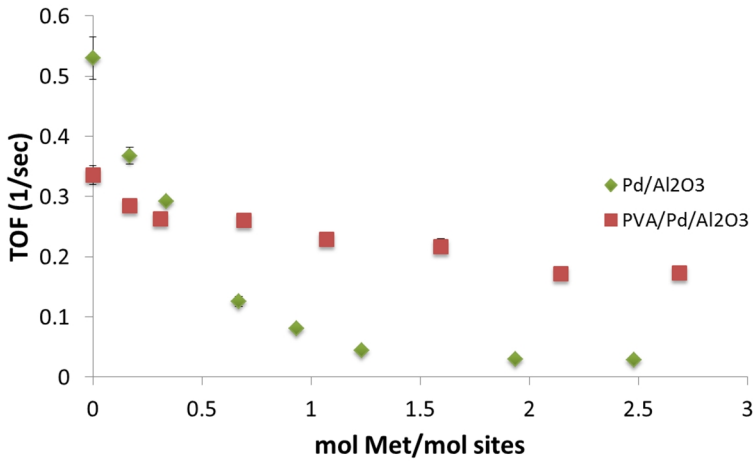


Figure 3. Influence of methionine on hydrogenation TOF for PVA overcoated catalyst. Reactions were carried out in stainless autoclaves at 50 °C with 27 bar H_2. TOF was measured between 10% and 20% conversion.

In the second prong of the approach taken, the use of bimetallic catalysts was explored as another means of making adsorption of amino acids energetically unfavorable. Appropriate alloying metals were chosen to decrease the size of extended palladium ensembles on the catalyst surface. Implicit in this decision was the assumption that adsorption of amino acids may require large ensembles of palladium atoms; reactions in the presence of methionine suggest that this may be the case. Further study would be needed to confirm this, however. While the TOF in the absence of methionine (0.075 sec^{-1}) is much lower than that of the base-case palladium catalyst (0.530 sec^{-1}), on a normalized basis the catalyst maintains more of its

activity in the presence of methionine (Figure 4). For these analyses, TOF is reported based on CO chemisorption. This is a measure of palladium sites only, as CO does not adsorb to tin.

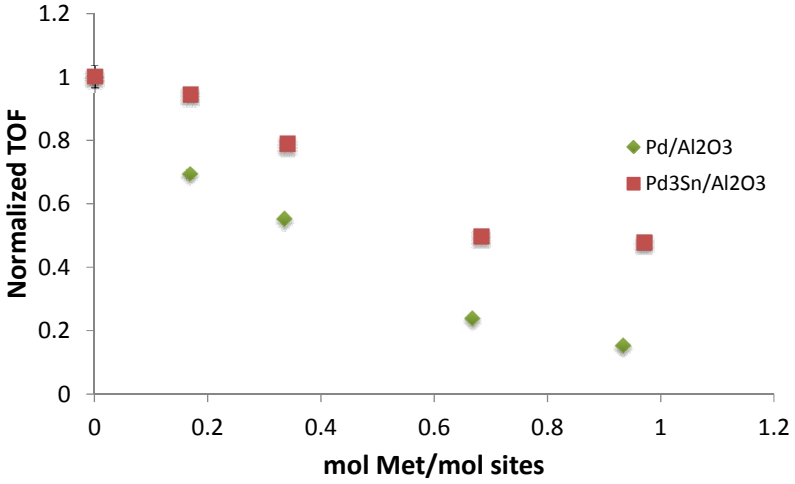


Figure 4. Influence of methionine on hydrogenation TOF for a Pd_3Sn alloy catalyst. Reactions were carried out in stainless autoclaves at 50 °C with 27 bar H_2. TOF was measured between 10% and 20% conversion.

Overcoating the Pd_3Sn catalyst with PVA yields a catalyst which is much more tolerant to amino acids than is the Pd_3Sn catalyst, as can be observed in Figure 5, again on a normalized basis. While the PVA-overcoated Pd_3Sn catalyst appears to be slightly more resistant than the simple PVA-overcoated palladium catalyst, the difference is far from dramatic. This suggests that, while the alloy does have an effect on amino acid adsorption, this is small relative to the impact of the polymer overcoating. However, the addition of the tin again results in a decrease in the hydrogenation rate in the absence of methionine (TOF of 0.139 sec^{-1}). The rate is less than half that of the overcoated palladium catalyst (TOF of 0.336 sec^{-1}); this suggests that the benefit of adding an alloying metal is outweighed by the resulting decrease in activity.

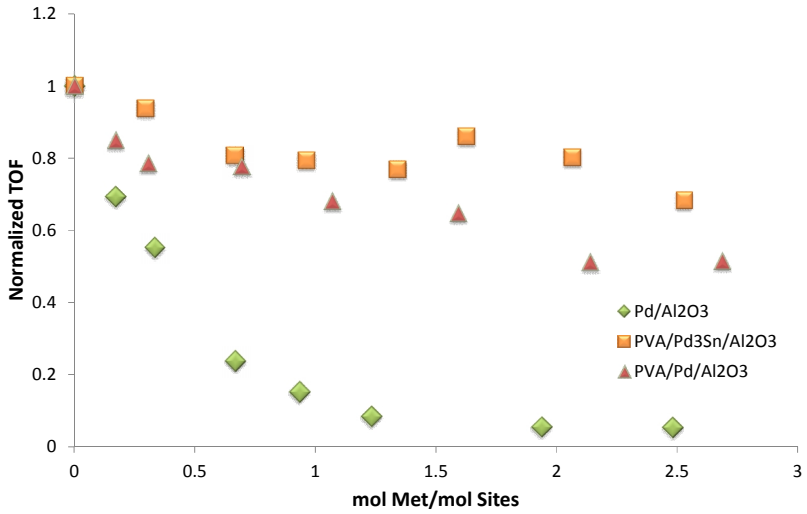


Figure 5. Comparison of PVA/Pd and PVA/Pd_3Sn catalysts. Reactions were carried out at 50 °C with 27 bar H_2. TOF was measured between 10% and 20% conversion.

The decreased activity in the absence of methionine is typical of several alloys investigated. Figure 6 shows TOF in the absence of methionine for several catalysts tested. While some are indeed more active that the Pd_3Sn alloy, none approach the activity of the PVA-overcoated palladium catalyst. Consequently, from the standpoint of achieving an active catalyst which is stable over a wide range of methionine concentrations, simple overcoating by poly(vinyl alcohol) is the optimal strategy.

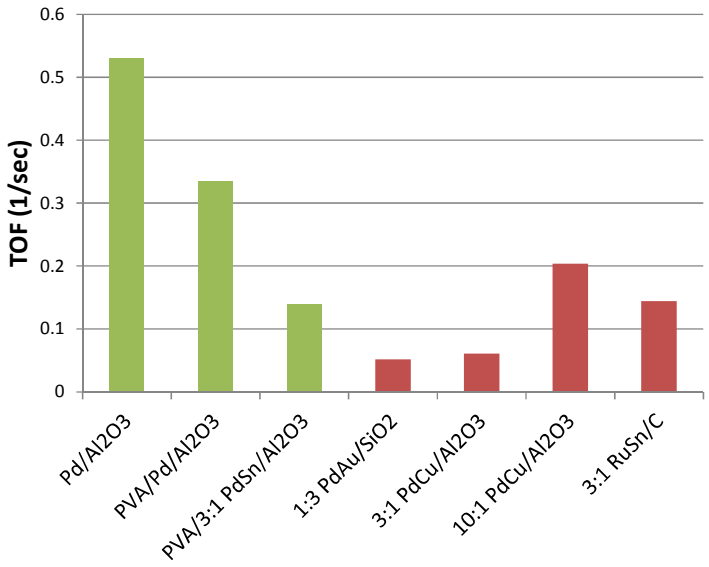


Figure 6. Comparison of TOF for several different alloy catalysts, all in the absence of methionine. Note that none exceeds the TOF for the PVA-overcoated palladium catalyst. Reactions were carried out at 50 °C with 27 bar H_2. TOF was measured between 10% and 20% conversion.

Several control experiments were performed in order to demonstrate that the mechanism by which PVA-overcoating imparts tolerance towards amino acids is indeed that of a solvation effect. If, rather than a solvation effect, a diffusion barrier selective for amino acids were at work, then equilibration of the catalyst and methionine prior to reaction would result in activity loss comparable to that observed for the non-overcoated catalyst. However, equilibration of the PVA/Pd_3Sn catalyst with methionine solutions for 12 hours at ambient temperature results in a TOF of 0.101 sec^{-1} for a Met:Sites ratio of 0.66:1, while the non-equilibrated reaction results in a TOF of 0.112 sec^{-1}. That the two TOFs are within experimental error implies that the benefit of PVA-overcoating is not that of a diffusion barrier. If the effect of overcoating were to simply remove methionine from the reaction mixture, a physical mixture of uncoated catalyst and supported PVA would display activity loss comparable with that of the overcoated catalyst. Thus, a similar experiment was performed equilibrating the feed solution, containing methionine again at a Met:Sites ratio of 0.66:1, with PVA-impregnated γ-Al_2O_3 for 12 hours. Following equilibration, the non-overcoated Pd/Al_2O_3 catalyst was added to the reactor and the reaction allowed to proceed as normal. In this case, the observed TOF was 0.160 sec^{-1}, which compares with the TOF of 0.123 sec^{-1} for the non-overcoated catalyst. Again, the closeness of these TOFs suggests that the PVA is not simply adsorbing the vast majority of the methionine in the reactor but, rather, preventing it from adsorbing on the catalyst surface.

The NMR investigation sought to complement the above work conducted in the Dumesic lab. In the Schmidt-Rohr lab, solid state ^{13}C NMR was used to gain insight about the mechanisms of methionine inhibition by characterizing molecular structures that are present on deactivated catalyst materials. Experimentally this was carried out through incorporation of uniformly ^{13}C labeled methionine into the reactors at the University of Wisconsin, and ^{13}C NMR characterization at the Schmidt-Rohr group at Iowa State University. ^{13}CNMR results of the deactivated catalyst materials show that a variety of chemical modifications have occurred to the methionine on these materials. Generally, the carbon spectra are dominated by alkyl and carboxylic acid groups, and exist in a distribution of structures. We see a reduction in the NCH abundance, which is consistent with elemental analysis data showing a 1:2 N:S molar ratio, instead of 1:1 in neat methionine (see Figure 7).

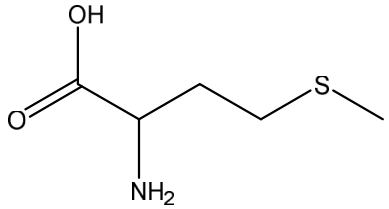
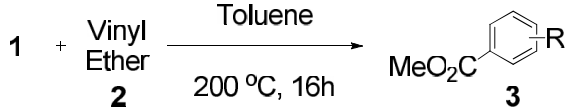
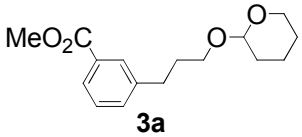
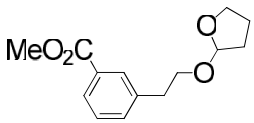
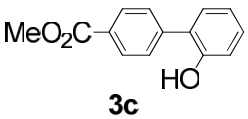
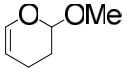
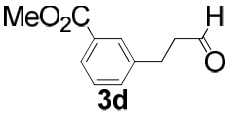


Figure 7. Methionine structure

The reactions of pyrones with alkenes has been studied in the Kraus lab. The reaction of pyrones with electron rich alkenes and captodative alkenes generates aromatic rings without the need for a catalyst. Additionally, the electron-donating group directs the regiochemistry of the cycloaddition, affording over 95% selectivity. Examples of this research are collated in the Tables below.

Table 1. Scope of reactions using vinyl ether dienophiles



Entry	Vinyl Ether	Aromatic Product	Yield (%)
1	 **2a**	 **3a**	77
2	 **2b**	 **3b**	61
3	 **2c**	 **3c**	77
4	 **2d**	 **3d**	77

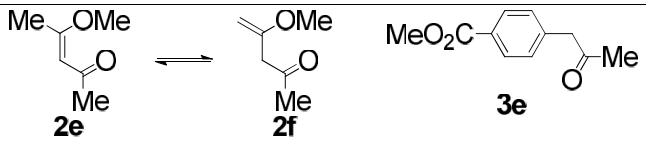
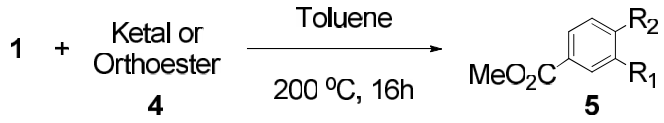
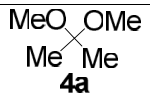
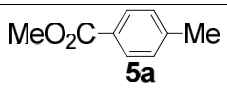
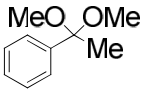
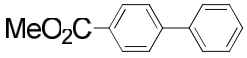
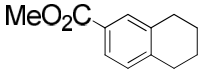
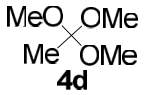
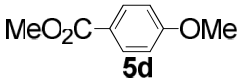
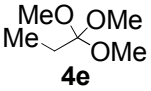
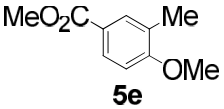
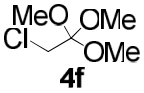
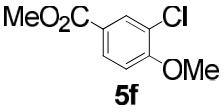
5	 **2e** **2f** **3e**		79

Table 2. Scope of reactions using ketals or orthoesters as dienophiles



Entry	Ketal or Orthoester	Aromatic Product	Yield (%)
1	 **4a**	 **5a**	89
2	 **4b**	 **5b**	85
3	 **4c**	 **5c**	81
4	 **4d**	 **5d**	73
5	 **4e**	 **5e**	94
6	 **4f**	 **5f**	72

Collectively, these tables show that a wide range of aromatic systems can be generated selectively in a single pot.

Other Relevant Work

The inhibitive effect of biogenic compounds on metal catalysts is vital in the Center's focus on upgrading biologically-derived compounds, and provides guidelines for the downstream purification of these compounds prior to catalytic upgrading. An understanding of the effect also places requirements on catalyst design for upgrading of biologically-derived feedstocks. To date, there are few examples in the literature[2,3] in this area; therefore the publication of our results will provide guidelines for the requirements of separation processes. There is also a dearth in the literature of catalysts which are resistant to inhibition by amino acids and other biogenic compounds. Publication of these results will represent a significant contribution, and may spark an interest in fundamental studies of catalyst inhibition by biogenic impurities that ultimately will result in further improvements in catalyst design.

Plans for the Next Five Years

Immediate plans revolve around demonstrating the time-on-stream stability of the PVA-overcoated palladium catalyst, both in the presence and absence of methionine. Additionally, *in situ* FTIR will be used in conjunction with ^{13}C solid state NMR and quantum chemical calculations to continue to develop a molecular picture of amino acids bound to the metal surface. *Operando* XAS may also be used to help elucidate this picture of catalyst inhibition and its effect on the hydrogenating metal. Other biogenic compounds will also be investigated as potential catalyst inhibitors.

Immediaate plans include extending the studies to nitrogen-containing functionality, generating bifunctional aromatics. This transformation will also be extended to a broader range of pyrone participants.

Expected Milestones and Deliverables

Time-on-stream stability of the PVA-overcoated catalyst will be assessed in the next quarter. FTIR and NMR experiments will be performed in the next year, as will quantum chemical calculations. If necessary, XAS experiments will follow these. The influence of other biogenic impurities will be investigated in parallel with spectroscopic studies.

Member Company Benefits

Members will have access to unpublished results from experimental studies.

Commercialization / Technology Transfer

TAL upgrading strategies have been disclosed to the Wisconsin Alumni Research Foundation (WARF), which is the technology transfer arm of the University of Wisconsin – Madison.

The Kraus group has submitted two disclosures to the Iowa State University Research Foundation regarding the terephthalic acid platform.

References

1. Chia, M.; Schwartz, T. J.; Shanks, B. H.; Dumesic, J. A., Triacetic Acid Lactone as a Biorenewable Platform Chemical. Green Chem. 2012, 14, 1850-1854.
2. Zhang, Z.; Jackson, J. E.; Miller, D. J., Effect of biogenic fermentation impurities on lactic acid hydrogenation to propylene glycol. Bioresour. Technol. 2008, 99 (13), 5873-5880.
3. Brands, D. S.; U-A-Sai, G.; Poels, E. K.; Bliek, A., Sulfur Deactivation of Fatty Ester Hydrogenolysis Catalysts. J. Catal. 1999, 186, 169-180.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.10 -** Selective Oxidation to Diacids

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Robert Davis	**02/5/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

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Statement of Project Goals

The goal of the work is to develop a mechanistic understanding of bifunctional alcohol oxidation. Factors that control the activity, selectivity, and stability of synthesized catalysts for the oxidation of model polyol compounds will be investigated. The project will use environmentally friendly reaction conditions. The alcohol oxidation will proceed at moderate temperatures and pressures in aqueous solution with dioxygen as the oxidant.

Project's Role in Center's Strategic Plan

One of the integrative test beds in the ERC is the production of bi-functional molecules from sugars. The number of biologically-derived products possible with enzyme bioengineering and microbial metabolic engineering is numerous. These biologically-engineered chemical products formed by Thrusts 1 and 2 include ring compounds, alcohols, aldehydes, olefins and acids. Homogeneous and heterogeneous catalysts can convert these biological products to a wide range of useful chemicals through decarbonylation, hydrogenation, hydrogenolysis, oxidation, cycloaddition and dehydration. In this project, oxidation of alcohols and aldehydes is being pursued to produce diacids, which are useful monomers in the plastics industry.

Fundamental Barriers and Methodologies

The fundamental barrier for the production of diacids is a lack of mechanistic understanding of bifunctional alcohol oxidation over supported metals. Neither the kinetics of alcohol oxidation nor the deactivation of a supported metal catalyst has an established mechanistic model. Thus, the barrier limiting the catalyst activity and selectivity or causing catalyst deactivation is not apparent and a catalyst cannot be designed to remedy the problem. What is known is that while the first oxidation of a terminal alcohol group to an acid happens readily over Au, Pt, and Pd, the subsequent oxidation of another terminal alcohol group on the same molecule has proven very difficult under conditions that are not highly basic.

The oxidation of glycerol (a model polyol) and hydroxymethylfurfural (derived from sugar) over supported Au catalysts in an aqueous solution effectively produces the hydroxy mono-acid at modest concentrations of base and produces di-acid at high concentrations of base. Unfortunately, supported Au catalysts do not effectively oxidize polyols without the addition of base. When supported Pt and Pd catalysts are used for glycerol oxidation in the absence of base, the hydroxy mono-acid is produced but the catalysts suffer from deactivation. Thus, methodologies have been developed to evaluate supported metal catalysts at acidic conditions in an experimentally consistent manner. The constant experimental conditions allow the comparison of a wide array of bifunctional alcohols to observe trends in oxidation activity and selectivity. In addition, the deactivation of supported Pt has been systematically explored to determine the cause of decreasing activity. Finally, bimetallic catalysts will be explored to determine if, as the literature suggests, these catalysts have superior activity.

Achievements

Last year, a procedure was developed to hold the pH of the reaction relatively constant by the addition of an organic acid. While the organic acid was determined to inhibit the alcohol oxidation rate by about 30% over Pt/C, the pH did not seem to have an effect on the rate of oxidation at pH's lower than neutral. The addition of 0.35 M acetic acid to hold the pH at about 2.5 was used throughout this study so that many different bifunctional alcohols could be compared on a consistent basis. The general scheme for α,ω-diol oxidation is shown in Figure 1.

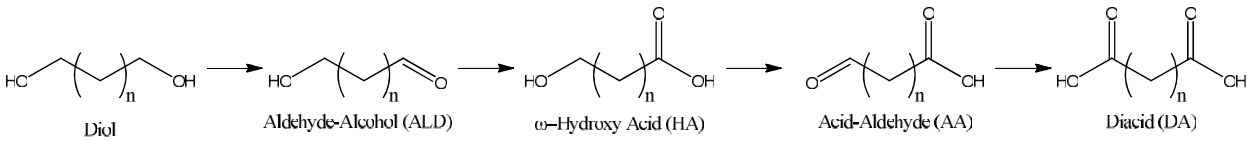


Figure 1. The general reaction pathway for α,ω-diol oxidation to the diacid with a carbon chain length between alcohol groups of (2+n)C.

Table 1. Effect of chain length for α,ω-diol oxidation over 2.69% Pt/C.

Substrate	TOF s^{-1}	Conv. %	Selectivity ALD	HA	AA	DA
1,2-ethanediol (EG)	0.03	14	0	100	0	0
1,3-propanediol (PDO)	0.05	20	35	54	0	11
1,4-butanediol (BDO)	0.15	16	91	9[b]	0	0
1,5-pentanediol (PEDO)	0.18	18	86	14	0	0
1,6-hexanediol (HDO)	0.19	20	94	6	0	0

Reaction conditions: 0.1 M substrate, substrate:Pt = 500:1 (mol:mol), T = 343 K, pO_2 = 10 atm, pH = 2.5. Conversion was reported at closest data point to 20% in order to compare selectivities at similar conversions. ALD = alcohol-aldehyde, HA = alcohol-acid, AA = aldehyde-acid, DA = diacid.

[b] HA selectivity for 1,4-butandiol oxidation was to γ-butyrolactone (GBL), not γ-hydroxybutyric acid (GHB). GBL and GHB have an equilibrium ratio of 68% of GBL and 32% GHB, but interconversion is slow. (Ciolino, 2001) Only GBL was observed by HPLC in this study.

Table 1 compares the TOF and selectivity for 2 carbon (2C) through 6 carbon (6C) α,ω-diol oxidation at identical conditions and a pH of 2.5 over Pt/C. The rate of oxidation was significantly higher for the 4C, 5C, and 6C diols compared to the 2C and 3C diols. The selectivity of the diol was primarily to the aldehyde (>80%) after 15 minutes of reaction, when the TOF was measured. When the selectivities are compared at similar conversion (~20%), the longer carbon chain diols (4-6) were primarily selective to the aldehyde-alcohol (ALD) product, while the shorter carbon chain diols (2-3) were selective to the γ-

hydroxy acid. Thus, the effect of the oxidation products was tested for 1,3-propanediol oxidation. However, the addition of equal molar ratios of either 3-hydroxypropionic acid or malonic acid had no effect on the initial oxidation rate of 1,3-propanediol

The oxidation of the α,ω-diols to the diacid was also investigated with the lower substrate to catalyst metal ratio of 100 (mol:mol). While 1,6-hexanediol can be oxidized to adipic acid with a selectivity of 85% at 100% conversion, diacid selectivity must be compared at similar conversions to compensate for the different rates of substrate oxidation. Figure 2 clearly illustrates that little to no diacid is observed for EG and PDO oxidation, while the longer carbon chain diols have significant selectivity at 90% conversion with the highest diacid selectivity of 62% for HDO to adipic acid.

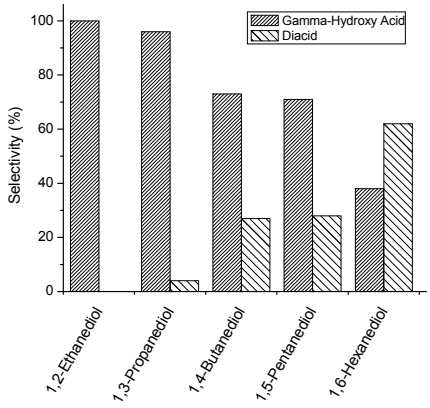


Figure 2. Acid selectivity at approximately 90% conversion for diol oxidation over Pt/C. Reaction conditions: 0.1 M substrate, substrate:Pt = 100:1 (mol:mol), T = 343 K, pO_2 = 10 atm, pH = 2.5. All carbon balances greater than 80%.

The chain length between alcohol groups appeared to be important for both initial oxidation activity and selectivity for α,ω-diols, which begs the question whether it is the proximity of the alcohol group or just the carbon chain length that determined the oxidation rate. Both mono-alcohols and various polyols were utilized for oxidation and the results are summarized in Table 2. The initial rate of reaction was significantly higher for mono-alcohols with two to four carbons in the chain compared to the α,ω-diols with the same amount of carbons. The increase in initial oxidation rate is especially pronounced for the two (400%) and three carbon (260%) substrates. These results suggest that the proximity of an alcohol group to another alcohol group was the determining factor for the decrease in initial oxidation activity. This is supported by the oxidation of 1,2-propanediol, 1,2-butanediol, and 1,3-butanediol, which have lower TOF's than their same carbon α,ω-diol counterparts. In addition, the sugar alcohol sorbitol had a significantly lower TOF than 1,6-hexanediol and appeared to deactivate the catalyst rapidly. However, 1,2-hexanediol appeared to have a TOF more similar to the faster α,ω-diols than to the other 1,2-diols. A molecule that is assumed to have a more stable alcohol and product is benzyl alcohol, which has the fastest TOF of all the substrates used in this work. While the trends of initial alcohol oxidation rate are not perfectly clear, the proximity of electron withdrawing groups does slow the TOF of a substrate.

Table 2. Oxidation of mono-alcohols and polyols over Pt/C.

Substrate	TOF (s^{-1})	Relative TOF to Same Carbon α,ω-Diol
Methanol (1C)	0.01	-
Ethanol (2C)	0.15	5
Propanol (3C)	0.18	3.6
Butanol (4C)	0.22	1.5
1,2-Propanediol (3C)	0.04	0.8
1,2-Butanediol (4C)	0.06	0.4
1,2-Hexanediol (6C)	0.14	0.7
1,3-Butanediol (4C)	0.07	0.5
Glycerol (3C)	0.06	1.2
Sorbitol (6C)	0.01	0.1
Benzyl Alcohol	0.32	-

Reaction conditions: 0.1 M substrate, substrate:Pt = 500:1 (mol:mol), T = 343 K, pO_2 = 10 atm, pH = 2.5.

The deactivation of supported Pt was systematically explored to determine the cause of the decrease in activity after the first hour of reaction. One possible reason for deactivation is the leaching of Pt metal into the acidic solution. However, elemental analysis of the filtered reaction medium after 1,6-hexanediol oxidation for 24 h showed that approximately 1.5% of the total available Pt metal leached. While leaching at this rate is an industrial concern, it is not likely the cause of the observed deactivation. A second possibility is the sintering of Pt particles during the reaction or leaching and re-deposition of Pt from solution that would increase the size of Pt particles. Characterization of the Pt particle size by TEM showed a negligible increase in particle size from 1.72 nm ± 1.20 nm before the reaction to 1.89 nm ± 1.45 nm after the reaction.

The over-oxidation of the supported Pt was also explored as a possible cause of deactivation. Over-oxidation can be defined as a high surface coverage of atomic oxygen or the formation of sub-surface oxygen caused by exposure to high pressures of dioxygen. The over-oxidation of Pt would prevent alcohol oxidation because reduced platinum is assumed to be the active site. The over-oxidation of Pt was purposefully performed by exposing the catalyst to dioxygen at 343 K for 2 h prior to 1,6-hexanediol oxidation. The dioxygen exposure did significantly decrease the TOF by 79%. However, if after the oxidation, the Pt catalyst was exposed to the alcohol in an inert atmosphere for just 30 min, a significant portion of the activity was recovered as seen in Table 3. The over-oxidation of Pt was found to be reversed by the alcohol. When the deactivated Pt/C was recovered and washed, though, the initial oxidation activity was significantly less than the fresh catalyst. This result suggests that while over-oxidation will deactivate the catalyst, the process is reversible, and that another source of deactivation must be poisoning the catalyst in the aqueous acidic environment.

Table 3. Inert startup, dioxygen startup, and recycle experiments with Pt/C for 1,6-hexanediol oxidation.

Experiment	$TOF_{1,6\text{-hexanediol}}$ (s^{-1})	% Decrease in Oxidation Activity
Inert Startup for 0.5 h (HDO in He)	0.19	-
Dioxygen Startup for 2 h then HDO	0.04	79
Dioxygen Startup for 2 h then Inert Startup for 0.5 h	0.15	21
Dioxygen Startup for 2 h then Inert Startup for 1 h	0.17	11
Wash Catalyst After 4 h Reaction then Inert Startup for 1 h	0.04	79

Reaction conditions: 0.1 M 1,6-hexanediol, 1,6-hexanediol:Pt = 500:1 (mol:mol), T = 343 K, pO_2 = 10 atm. TOF_{HDO} = [mol 1,6-hexanediol converted (mol $Pt_{surface})^{-1}$ s^{-1}]. TOF_{HDO} was based on 1,6-hexanediol conversion at t = 15 min. The % decrease in oxidation activity = [(TOF_{HDO} w/inert startup − TOF_{HDO} experiment)/TOF_{HDO} w/inert startup] × 100

The production of CO and CO_2 has been observed in electro-oxidation and oxidation of alcohols over supported Pt in organic solvents, thus, it was hypothesized that CO might be poisoning the Pt surface. While it might be slow at such low temperatures, CO oxidation is possible over supported Pt in the presence of oxygen. Thus, the amount of CO_2 found in the headspace of the batch reactor after 20 h of reaction was measured by a thermal conductivity detector with gas chromatography. The production of CO_2 was measured for all alcohol substrates. Interestingly, the amount of CO_2 produced from ethylene glycol and 1,3-propanediol was significantly higher than ethanol or 1,6-hexanediol. A hypothesis is that decarbonylation of the aldehyde was responsible for the formation of CO on the platinum surface and its oxidation produced CO_2. This would appear to support the fact that the oxidation TOF of 1,6-hexanediol and ethanol is much faster than ethylene glycol and 1,3-propanediol. The CO strongly adsorbed on the surface could explain catalyst deactivation, but also why the catalyst never deactivates completely, as the CO oxidation rate would limit the alcohol oxidation rate. To observe if CO is produced under the reaction conditions, in-situ ATR-FTIR experiments were performed with a 2% Pt/SiO_2 (Pt dispersion = 0.65) dip coated onto a ZnSe IRE. The oxidation of a 0.1 M benzyl alcohol solution was investigated in a flow reactor setup at a flow rate of 1 cm^3 $min^{-1}$and 3 bar of pressure at room temperature.

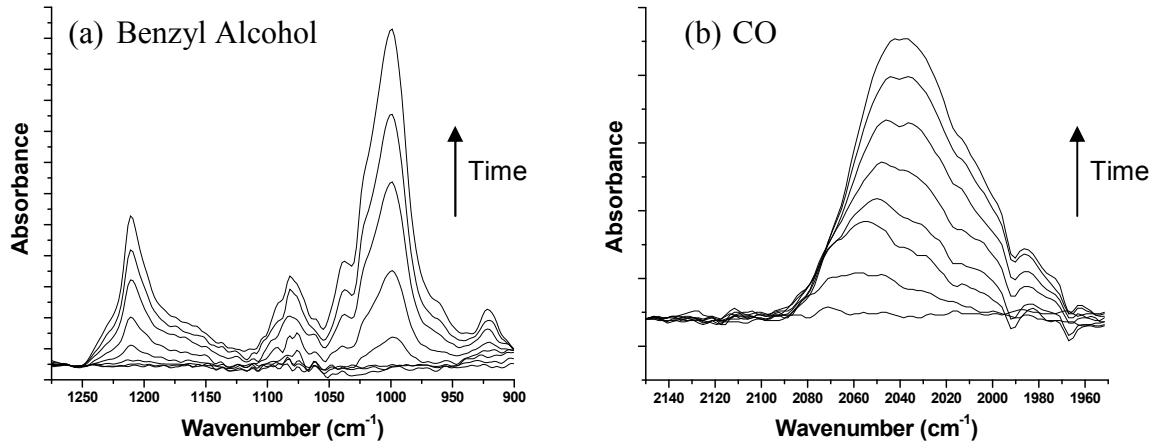


Figure 3. The appearance of (a) benzyl alcohol and (b) CO after flowing 0.1 M aqueous solution of benzyl alcohol saturated with N_2 over Pt/SiO_2 at 3 atm N_2 and 298 K.

Figure 3 shows benzyl alcohol (a) appearing and simultaneously CO (b) appearing adsorbed to the Pt surface at 2042 cm^{-1} in an inert atmosphere. The CO observed on the Pt surface increased with time to an eventual maximum after 20 min. This work approximately mimics the conditions of the semi-batch experiments during the inert startup. Most likely, some alcohol is dehydrogenated to an aldehyde and the aldehyde is decarbonylated to CO that is strongly adsorbed to Pt before the reaction is "initiated" by the addition of dioxygen.

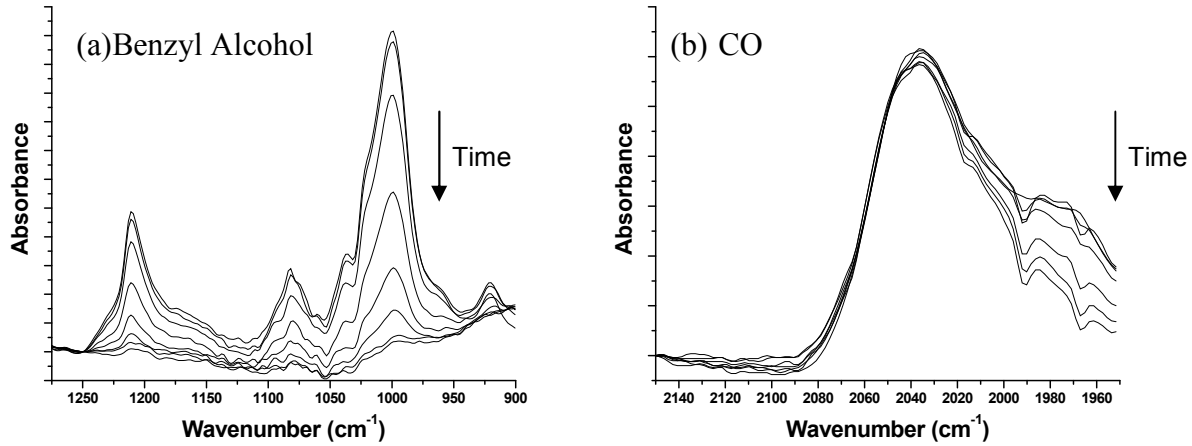


Figure 4. The removal of (a) benzyl alcohol and (b) the strong adsorption of CO after flowing H_2O saturated with N_2 over Pt/SiO_2 at 3 atm N_2 and 298 K.

The removal of benzyl alcohol from the flow reactor by flowing nitrogen saturated water is shown in Figure 4 as benzyl alcohol (a) disappears from the cell. However, CO (b) is not removed from the sample and is strongly adsorbed to Pt. Upon the addition of O_2 to the sample cell, however, CO is rapidly removed as shown in Figure 5. The strongly adsorbed CO is quickly oxidized on Pt when dioxygen-saturated water flowed over the sample. To draw a correlation between the flow reactor and the semi-batch reactor, any CO that was adsorbed on the Pt surface either during the inert startup or during the reaction is rapidly oxidized when the catalyst is exposed to dioxygen. This is most likely the route to produce CO_2 at these reaction conditions.

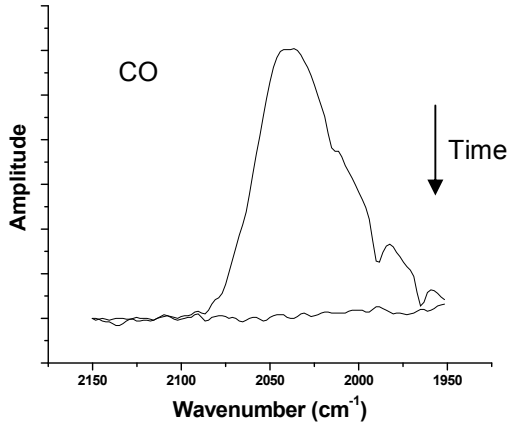


Figure 5. The rapid removal of CO after flowing H_2O saturated with O_2 over Pt/SiO_2 at 3 atm and 298 K.

The synthesis of bimetallic PtAu nanoparticles supported on activated carbon was performed in a project started with an NSF REU student Jason Bates from the University of Kansas. A 1.1% Pt-Au/C catalyst with a Pt/Au atomic ratio of 0.86 had a significantly higher TOF for 1,6-hexanediol oxidation of 0.68 s^{-1} compared to the 0.20 s^{-1} TOF of monometallic 0.73% Pt/C catalyst prepared similarly. The catalysts were synthesized by the sol-immobilization method where the nanoparticles were formed in solution using PVA as a stabilizer and then deposited onto the activated carbon support. The removal of the PVA stabilizer was determined to increase the rate of oxidation by over 70%.

The particle size for the Pt-Au/C catalyst was determined to be 4.1 ± 1.6 nm by TEM shown in Figure 6. The EDS spectra of individual particles all indicated the presence of both Pt and Au and the composition was fairly consistent when EDS was performed over several hundred particles, a few dozen particles, or single particles. The diffraction peak of the bimetallic catalyst from XRD fell between the monometallic peaks at 38.8°, which suggests that a significant portion of the metals were alloyed.

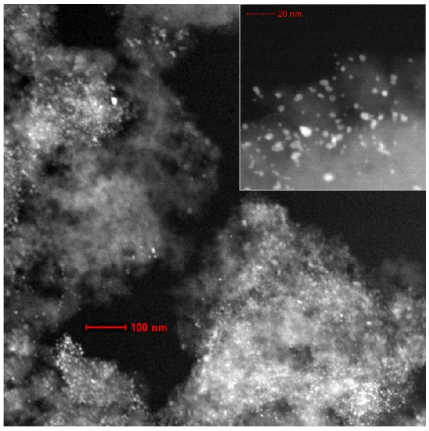


Figure 6. TEM dark field images of 1.1% Pt-Au/C showing disperse nanoparticles.

When elemental analysis of the filtrate was measured by ICP-AES, no leaching of Au or Pt was found after 24 h of reaction with the Pt-Au/C catalyst. Figure 7 depicts the reaction profile for both the Pt/C and Pt-Au/C catalysts for 1,6-hexanediol oxidation. Despite the initial increase in oxidation rate for 1,6-hexanediol over the Pt-Au/C catalyst, the catalyst deactivated severely after just 15 minutes. It is unclear whether the deactivation is similar to the monometallic Pt/C catalyst or is fundamentally different at the beginning of the reaction.

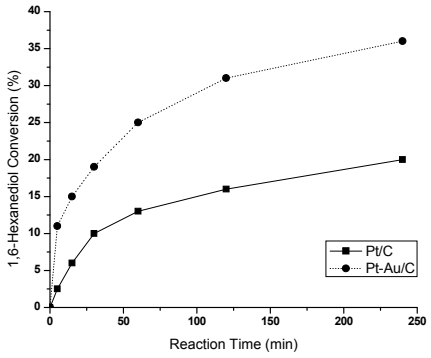


Figure 7. Reaction profile for the oxidation of 1,6-hexanediol over Pt/C and Pt-Au/C. Reaction conditions: 0.1 M 1,6-hexanediol (HDO), HDO:Met. ~ 500, pO_2 = 10 bar, T = 343 K, 0.35 M acetic acid, pH = 2.5.

Plans for the Next Five Years

The selective oxidation of bifunctional molecules to diacids project has established that the production of diacids is possible at acidic conditions and the rate of oxidation depends on the type and number of functional groups present in the molecule. Plans for the next few years include developing a mechanistic model for alcohol oxidation in aqueous solution based on reaction kinetics, further investigation of deactivation of platinum during alcohol oxidation, and determining the effectiveness of PtAu bimetallic nanoparticles for alcohol oxidation.

Expected Milestones and Deliverables

The project has been presented at the 2012 American Institute of Chemical Engineers national meeting and has a manuscript in preparation for submission to the Journal of Catalysis. In addition, the research will be presented at the 23rd North American Catalysis Society Meeting and the 10th Congress on Catalysis Applied to Fine Chemicals in Turku, Finland. All future catalyst compositions, reaction rates, and performance evaluations for bifunctional alcohol oxidation will be reported to the center.

Member Company Benefits

Members will have access to all experimental studies of selective oxidation reactions by supported metal catalysts. The member companies will also have access to catalyst synthesis and characterization techniques for novel supported metal catalysts.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.11** - Migration of Functional Groups

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
L. Keith Woo	**02/12/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: L. Keith Woo

Graduate Student: Gina M. Roberts, Iowa State University

REU Undergraduate: Philip Pierce, Virginia Polytechnic Institute

Statement of Project Goals

Organometallic strategies will be applied to the development of a catalytic toolbox for efficient conversions of renewable materials into chemical replacements for petroleum-based feedstocks.

Project's Role in Center's Strategic Plan

A key objective in this undertaking is to develop efficient and robust catalysts for the conversion of biosynthetic, short chain unsaturated fatty acids into bifunctional commodity chemicals. Representative targets include organic diacids. These dicarboxylic acids are useful precursors for the pharmaceutical and food industries. For example, preparation of fragrances, polyamides, adhesives, lubricants, and polyesters are some of the key applications of diacids.

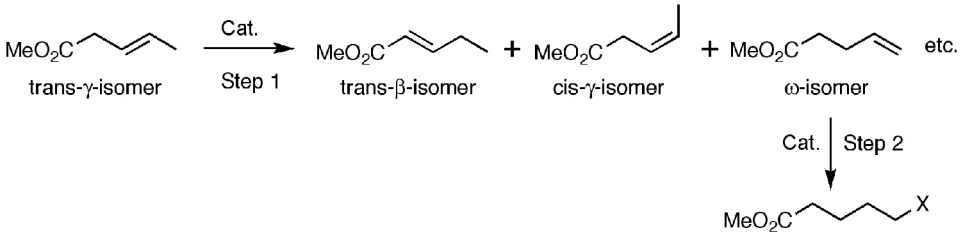
Fundamental Barriers and Methodologies

The efficient conversion of renewable natural resources, such as fatty acids, into useful industrial chemicals is an important means of reducing our dependence on crude oil. A goal of this project is to transform unsaturated fatty acids into value-added compounds. Potential targets are monomers for producing useful materials, such as polyesters, nylons, fragrances, and flavors. This would be a major step towards replacing petroleum products with biorenewable resources. For example, the industrial synthesis of adipic acid, a key monomer for producing nylon-6,6, is derived from benzene, a petroleum refinery commodity chemical. The project goal involves developing catalysts or catalyst systems that are capable of transforming monounsaturated fatty acid esters into derivatives with another functional group at the terminal (ω) carbon of the fatty acid. The double bond in the initial substrate may be anywhere along the carbon backbone. Moreover, if the biocatalysts in Thrust 2 produce a hydroxylated fatty acid, these compounds can be dehydrated to an unsaturated acid and esterified to provide the renewable feedstock for our chemical catalysts.

Current technology to convert unsaturated fatty acid esters involves a palladium catalyst that requires an expensive, air-sensitive phosphine ligand, high pressures of carbon monoxide, and high temperatures (130 ^{o}C). Our approach for addressing these issues is to replace the

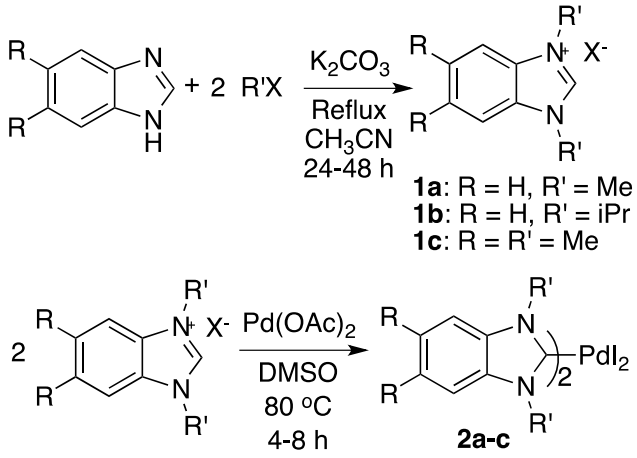
phosphine ligand with N-heterocyclic carbenes, NHCs. These NHCs have similar characteristics to phosphines, but are relatively easy to prepare, have tunable bulkiness and electronic properties, and are more robust that phosphines.

The catalysis approach that we are developing involves a one-pot, two-step process. In the first step, the double bond of the unsaturated fatty acid ester is rapidly isomerized along the hydrocarbon chain to produce a pool of all possible positional isomers. A second reaction only occurs when the double bond moves into the terminal position of the hydrocarbon chain. The selectivity of the second step results in a dynamic resolution of the mixture produced in step 1 such that only one double bond isomer is converted to product. The two reactions continue in tandem until the interconverting pool of double-bond isomers is transformed into one pure compound.

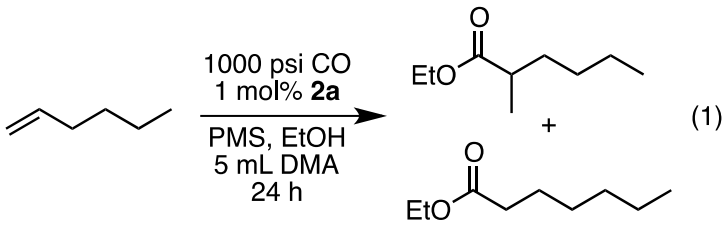


Achievements

Synthesis of the N-heterocyclic carbene (NHC) ligands was accomplished by alkylation of benzimidazole. The resulting benzimidazolium salts were readily metallated by the addition of



palladium acetate, $Pd(OAc)_2$, in the presence of base. The three Pd NHC complexes, **2a-c**, were evaluated for their activity as catalysts for the esterification of olefins (alkoxycarbonylation) in the presence of ethanol and carbon monoxide. Strong acid additives, such as methylsulfonic acid (MSA), led to the decomposition of the catalyst, presumably due to protonation of the NHC ligand and its removal as an imidazolium salt. However, weaker acids such as pyridinium methylsulfonate (PMS) successfully resulted in the conversion of olefins to esters. Of the three catalysts, the benzimidazolidine complex, **2a**, was extremely efficient at the esterification of 1-hexene (eq. 1), with yields as high as 88% and branched to linear (b:l) ratios of 2:1 (Table 1).



(1)

Table 1. Optimization of Alkoxycarbonylation Conditions for catalyst **2a**.

Entry	% PMS	EtOH (mmol)	1-hexene (mmol)	T (^{o}C)	Yield (%)	Ratio b:l
1	5	14	56	100	32	2.3
2	10	14	56	100	44	2.4
3	15	14	56	100	58	2.4
4	10	14	56	110	80	1.8
5	15	14	56	110	88	2.0
6	15	14	14	110	22	2.1
7	15	56	14	110	6	2.1

Conditions: 1 mol% **2a** and 5 mL DMA, 1000 psi CO, 24 h.

In examining the nature of the catalyst, it was found that alkoxycarbonylation did not occur in the absence of NHC ligands. For example, alkoxycarbonylation was ineffective with PMS and Pd reagents such as $Pd_2(dba)_3$ or $Pd(OAc)_2$, under similar conditions that gave high conversion with **2a**. However, $Pd(OAc)_2$ became active for carbonylation (32% yield of products) in the presence of **1a** (15 mol%), producing a nearly 1:1 ratio of branched to linear products. Catalyst **2a** also remained active when PMS was replaced with a similar loading of dimethylbenzimidazolium iodide (**1a**), resulting in a 95% yield of products and an improved linear selectivity (b:l = 1:1). The presence of Pd was necessary as salt **1a** alone was inactive for carbonylation in the absence of a palladium source.

Improvement to the linear selectivity by replacing the acid additive PMS with **1a** suggested that altering the acid source might be key to catalytic performance. On examining the influence of the pyridinium acid structure, it was found that neither the presence of a single methyl group on the pyridinium ring (2-picolinium, 4-picolinium) nor the inclusion of 2,6-methyl groups (lutidinium) resulted in a significant change in product selectivity. Expanding the range of acids to include Lewis acids ($ZnCl_2$, Ph_3B), nonanoic acid, and sulfonic acids (MSA; p-toluene sulfonic acid, PTSA), resulted in little improvement to selectivity or yield. Reevaluation of the catalytic system revealed that 100% alkoxycarbonylation could be obtained using catalyst **2a** without any acid additive.

Pd-NHC-catalyzed alkoxycarbonylation of additional olefins generally resulted in quantitative

conversion of terminal olefins to ester product (Table 2). It is also important to note that alkoxycarbonylation of internal olefins was dramatically improved over systems that included an acid additive such as PMS. When using compounds containing vinylic functionality (e.g. styrene and ethyl acrylate), a large amount of polymeric material was formed.

Table 2. Alkoxycarbonylation of Various Olefins With Catalyst **2a**.

Entry	Substrate	Acid	Yield % (b:l)
1	1-pentene	PMS	85 (1.8)
2	1-pentene	-	99 (1.6)
3	1-octene	-	100 (1.6)
4	cyclohexene	PMS	16
5	cyclohexene	-	83
6	ethyl 4-pentenoate	-	100 (2.1)
7	styrene	PMS	27 (2.2)[a]
8	ethyl acrylate	PMS	31[a]

Conditions: 14 mmol EtOH, 56 mmol olefin, 1 mol% **2a**, 15 mol% PMS where noted, 5 mL DMA, 1000 psi CO, 110 ^{o}C, 24 h. [a]Large amount of polymer product was detected.

SUMMARY

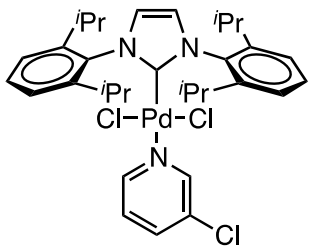
We have shown that Pd benzimidazolylidene complexes are robust and high-yielding alkoxycarbonylation catalysts in the absence of acid additives. While many reports indicate analogies between phosphines and NHC ligands, it is evident from our preliminary work that Pd-NHC alkoxycarbonylation systems are quite disparate from those of Pd-phosphines. The nature of the active Pd intermediates remains unclear, but there is strong evidence that the NHC is vital to reactivity.

Other Relevant Work

We are studying the use of surfactants for performing Pd-mediated Sonogashira reactions in water as a green approach for catalysis. Our results show that simple and inexpensive surfactants such as SDS (sodium dodecylsulfate) or CTAB (cetytrimethylammonium bromide) used with $Pd(PPh_3)_2Cl_2$ is an effective aqueous, catalytic system for the coupling of aryl bromides or iodides with terminal acetylenes.

Plans for the Next Year

Work on optimizing catalysts for producing bifunctional chemicals will continue with the goal of addressing several issues. Although our Pd-NHC catalysts are capable of esterifying C-C double bonds in high yields, the regiochemistry currently favors the internal isomer over the desired terminal isomer. Control over the isomer selectivity should be possible by increasing the steric size of the NHC ligand so that the less bulky terminal product is preferred. Thus, larger nitrogen substituents, such as 2,6-diisopropylphenyl, will be introduced into the NHC ligand.



Identifying reaction intermediates will be undertaken as a means to improve our catalytic system. In addition, we will also begin to examine other modifications such as replacing the alcohol reagent with amines. This should allow us to introduce nitrogen functionality into the product and represents a novel approach to generating amides from biomass feedstocks.

Expected Milestones and Deliverables

New transition metal catalysts based on N-heterocyclic carbene ligands will be designed and optimized for the transformation of unsaturated fatty acid methyl esters (FAMES) into industrially useful bifunctional molecules. A key target will be the catalytic conversion of biologically derived short chain unsaturated FAMES into α,ω-diesters. These bifunctional molecules can serve as biorenewable replacements for petroleum-derived adipic acid in the industrial production of nylons. A manuscript on our initial Pd-NHC alkoxycarbonylation catalyst has been accepted by the American Chemical Society journal *Organometallics*. A second submittal that describes the elaboration of this system is under preparation.

Member Company Benefits

Member companies will have access to results on catalyst development for the conversion of unsaturated fatty acid methyl esters into α,ω-difunctionalized products.

Commercialization / Technology Transfer

If successful and applicable, the catalyst technology will be patented and offered for licensing.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **02-12F2 -** Production of Monomers for Nylon-6,6 from Biorenewable Sugars

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Brent Shanks	**02/20/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Graduate Students: Tianfu Wang and Mike Nolan, Iowa State University; Matt Ide, University of Virginia
Faculty Advisors: Brent Shanks, Iowa State University; Robert Davis , University of Virginia

Statement of Project Goals

This project aims at designing a novel catalytic toolkit that could be exploited to convert biorenewable sugars to replace petroleum feedstocks for the production of key monomers used for nylon-6,6 synthesis.

Project's Role in Center's Strategic Plan

Several parallel research efforts using glucose as feedstock are currently under investigation in the center. In this context, the combination of different reaction schemes such as dehydration, oxidation, and amination could lead to the synergistic delivery of the monomer needed in commodity chemical synthesis, such as nylon 6,6.

Fundamental Barriers and Methodologies

To integrate and coordinate the effort across the two institutions, the project entails five steps: First, the dehydration of glucose to 5-hydroxymethyl furfural (HMF). Second, the dehydration of 1,2,6-hexanetriol to 1,6-hexanediol. Third, the conversion of 1,6-hexanediol to hexamethylenediamine. Fourth, the oxidation of 1,6-hexanediol to adipic acid. And finally, adipic acid and hexamethylenediamine will be polymerized to nylon-6,6 using established technology. The first and third steps will be carried out by Tianfu Wang at Iowa State University, the second step will be carried out by Michael Nolan at Iowa State University. The fourth and fifth steps will be carried out by Matt Ide at the University of Virginia.

Achievements

The conversion of 1,6-hexanediol to hexamethylenediamine starts from hexanediol in the presence DIH as oxidation and ammonia as the nitrogen source followed by hydrogenation to the dinitrile catalyzed by Raney Ni. As shown in Figure 1, the diol could be converted to the adiponitrile in yield of greater than 80% and subsequently, the adiponitrile could be hydrogenated in the presence of Raney Ni catalyst in yields of greater than 90%. Thus, overall molar yield of the diamine from

the diol could be greater than 70%.

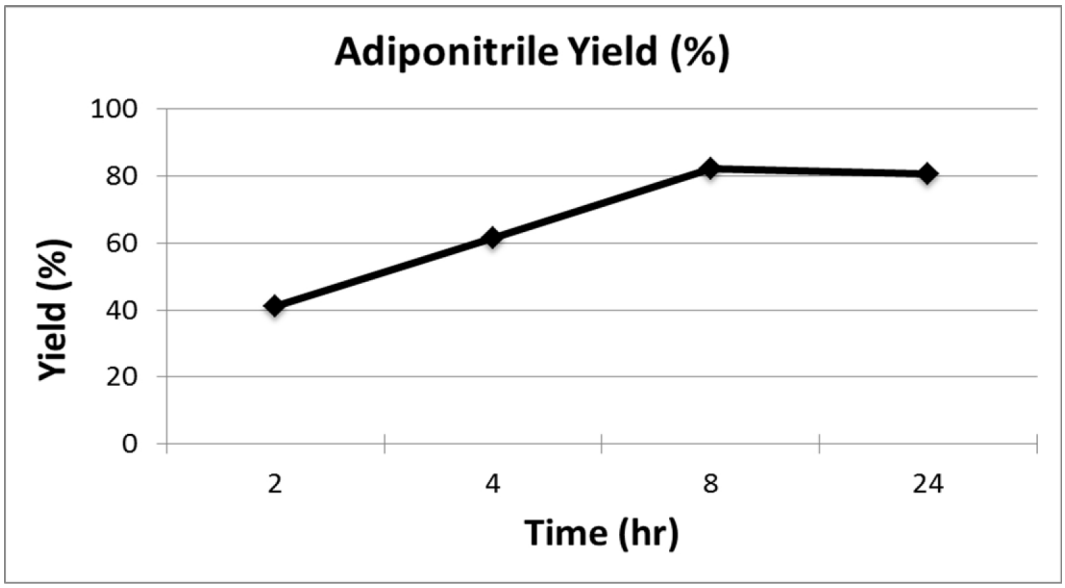


Figure 1. Adiponitrile yield with time Raction condictions: starting materials of 1,6-hexanediol, DIH, and ammonia.

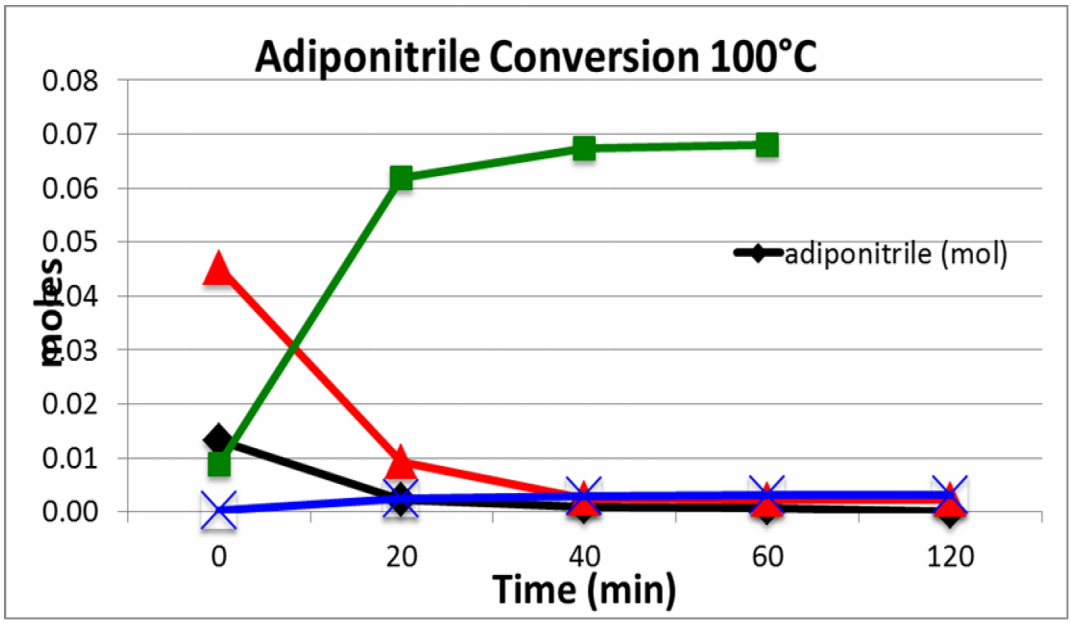


Figure 2. Adiponitrile hydrogenation to the 1,6-hexanediamine, reaction temperature of 100 degree C and catalyst of Raney Ni

The oxidation of 1,6-hexanediol to produce adipic acid with dioxygen was performed over two different catalysts in an aqueous solvent. An activated charcoal supported Au catalyst was synthesized by the sol immobilization method. The nanoparticles were formed in an aqueous solution using polyvinyl alcohol as a stabilizer and then reduced by sodium borohydride. The resulting gold nanoparticles were then deposited on the activated charcoal support. The 3% Au/AC catalyst was then reduced at 573 K for 2h with 100 cm^3 min^{-1} of flowing H_2 to remove the polyvinyl alcohol from the catalyst. The oxidation of 0.1 M 1,6-hexanediol was performed for 24 h at 343 K with 10 atm dioxygen and 1 M sodium

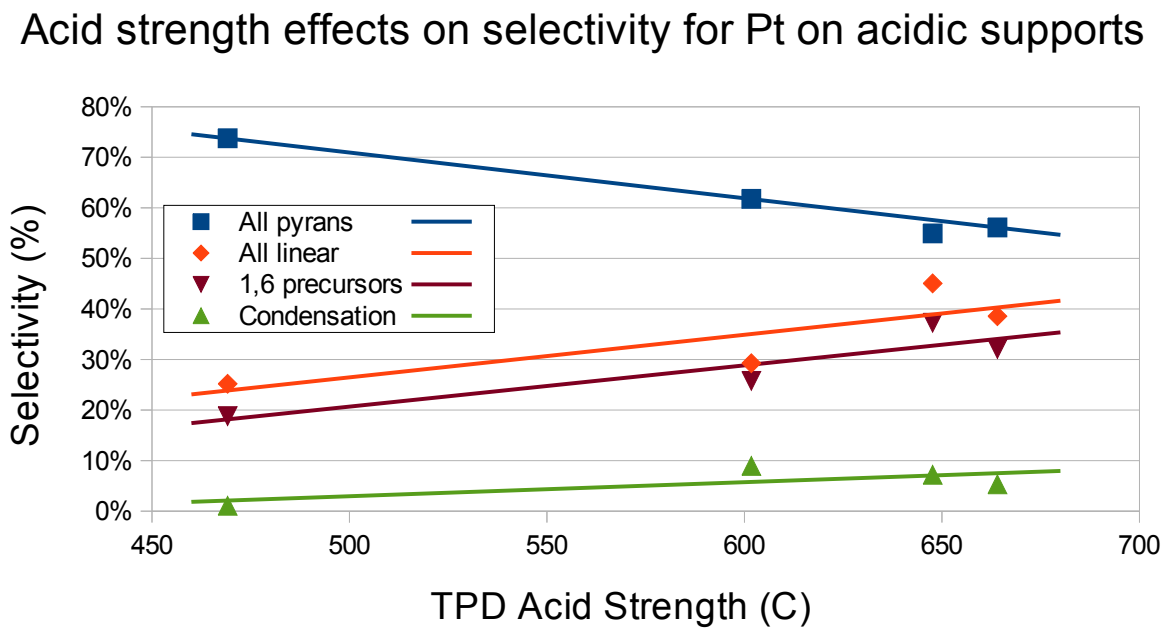
hydroxide. The yield of adipic acid from 1,6-hexanediol at these conditions was approximately 97%. The use of a homogeneous base, however, necessitates that the product solution be ion exchanged before use to produce free acids rather than salts. Thus, acidic conditions were evaluated for adipic acid production.

A second catalyst was also used for the production of adipic acid at acidic conditions. A titania supported Pt catalyst was synthesized by incipient wetness impregnation. The 3% Pt/TiO_2 catalyst was then calcined at 673 K for 4 h with 100 cm^3 min^{-1} of flowing air followed by being reduced at 573 K for 2h with 100 cm^3 min^{-1} of flowing H_2. The chemisorption of the Pt/TiO_2 catalyst with dihydrogen was performed at 303 K and the dispersion was determined to be 0.63 with an approximate particle size of 1.6 nm. The oxidation of 1,6-hexanediol was performed for 24 h at 343 K with 10 atm dioxygen without any added base and the yield of adipic acid was determined to be 89%. While the yield was slightly lower than that produced at basic conditions, the free acid was produced without any need for costly separations by ion exchange.

Finally, the production of nylon-6,6 was performed by the step-growth condensation polymerization of hexamethylenediamine and adipic acid. A solution of 2 g of adipic acid and small amount of thionyl chloride were added to a cyclohexane solvent and kept at approximately 333 K for 1 hour. A solution of aqueous hexamethylenediamine was made by adding an equimolar amount of the polyamide and a small amount of sodium hydroxide. The polymerization was initiated by adding the cyclohexane solution to the aqueous solution and the pH was adjusted to be greater than 7 by the addition of a 10% sodium hydroxide solution. The nylon-6,6 polymer was removed from solution and all solid recovered was washed with distilled, deionized water and dried overnight in air at 333 K. The recovered polymer was weighed and a yield of approximately 70% based on weight was achieved.

Building on previous work of dehydration over solid acids, the dehydration of 1,2,6-hexanetriol was carried out over platinum supported on solid acid catalysts. Work on solid acids had found that selectivity to precursors of 1,6-hexanediol had a "ceiling" at about 30% because of a tradeoff between catalysts being selective to either pyran ring products in the case of low-strength acids, or to condensation of linear products in the case of strong acid catalysts. In the case of these supported catalysts, we wanted to see if the selectivity ceiling could be cleared by hydrogenating 6-hydroxyhexanal to 1,6-hexanediol during the reaction.

Catalysts were prepared by supporting 1 wt.% of platinum on amorphous silica-alumina, Y-zeolite, or H-ZSM5 (all 80:1 Si:Al ratio), or on amorphous niobia. Catalyst acid strengths were determined from temperature programmed desorption of ammonia. Catalyst selectivity was determined by flowing 1,2,6-hexanetriol in a stream of flowing atmospheric-pressure hydrogen over a fixed bed of a given catalyst, and the products collected and analyzed by GC-MS.



Initial results from the dehydration reaction have found that increasing acid strength correlates with decreasing selectivity to pyran rings and increasing selectivity toward linear products. Also, unlike the case for solid acids, selectivity toward condensation does not make up a large fraction of the linear products, allowing for higher selectivities toward precursors of 1,6-hexanediol. Mechanistically, these results suggest that the precursors to 1,6-hexanediol produced by dehydrating 1,2,6-hexanetriol, namely 6-hydroxyhexanal, tend to condense at reaction conditions, but that the condensation reaction is suppressed upon the addition of metal.

Expected Milestones and Deliverables

The integration of the three graduate students' research will lead to a proof-of-concept for the production of nylon from biorenewable sugars.

Member Company Benefits

The successful demonstration and technical development of nylon synthesis will potentially open a new avenue for our member companies to leverage biomass to use as their monomers.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **02-12F3 -** Applicability of Novel Heterogeneous Palladium Catalysts in Industrially-Relevant Organic Transformations

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
George Kraus	**02/18/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Graduate Students: Jennifer Lee, Iowa State University; Angelica Benavidez and Jonathan Paiz, University of New Mexico

Faculty Advisors: Drs. George A. Kraus, Iowa State University and Abhaya Datye, University of New Mexico

Statement of Project Goals

In addition to the palladium catalyzed reaction that produces alpha olefins, the proposed project aims to explore industrially-relevant reactions involving a catalytic palladium system including the Heck and Suzuki couplings. Since these reactions are common in industry, developing a new heterogeneous catalyst system by loading palladium nanoparticles onto a suitable support would be ideal. We will work to improve yields over current systems.

Project's Role in Center's Strategic Plan

Catalyst development is a key element in the plan for Thrust 3. The applications of the catalysts developed by the Datye group to industrially significant palladium mediated reactions will further the mission of Thrust 3.

Fundamental Barriers and Methodologies

Replacement of homogeneous catalysts with heterogeneous catalysts is a significant objective of industrial ecologists. The heterogeneous catalysts developed by the Datye group represent a significant advance.

Foreign Collaborations

None.

Achievements

We synthesized a 5wt% Pd on Vulcan carbon sample using the direct alcohol reduction method. Scanning transmission electron microscopy (STEM) was used to characterize this and a commercially available sample commonly used for these reactions. Figure 1a shows the sample synthesized at UNM and Figure 1b shows a commercially available sample. The Pd nanoparticles in the commercially available sample are significantly larger, ~2nm in diameter, compared to the sample we synthesized which were on average less than 1nm in diameter.

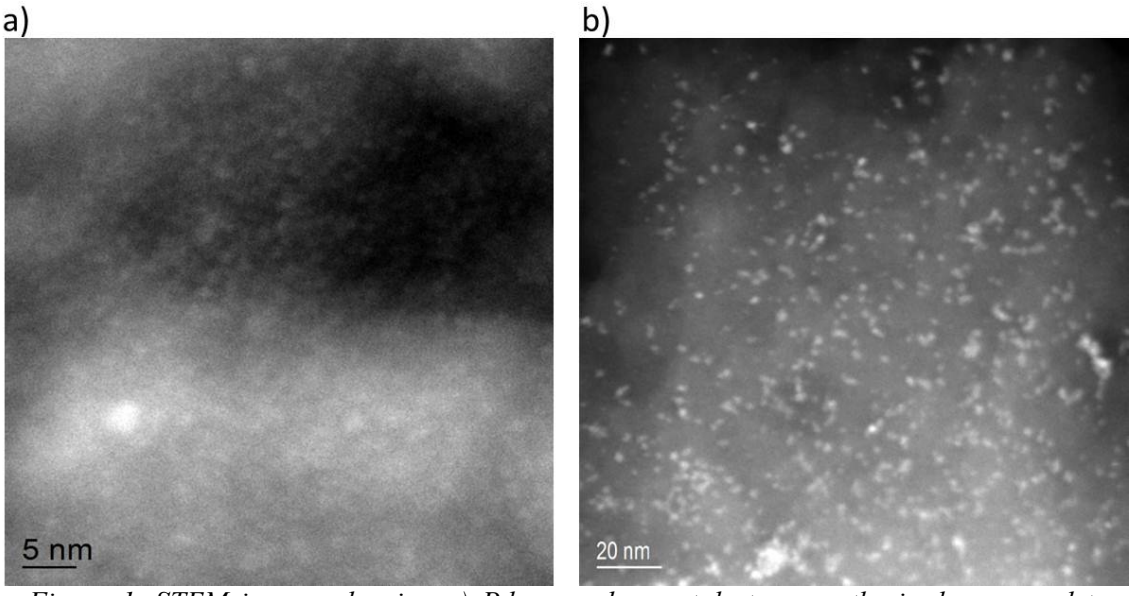
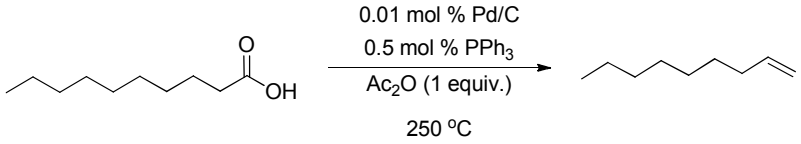


Figure 1. STEM images showing a) Pd on carbon catalyst we synthesized compared to b) Pd on carbon sample that was commercially obtained.

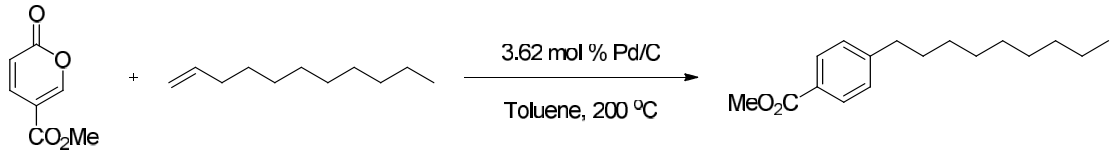
We compared the results from a commercially obtained palladium on carbon with the palladium on carbon prepared by the Datye group for the following reactions, which incorporated the use of a heterogeneous catalyst.

1. Generation of α-olefins



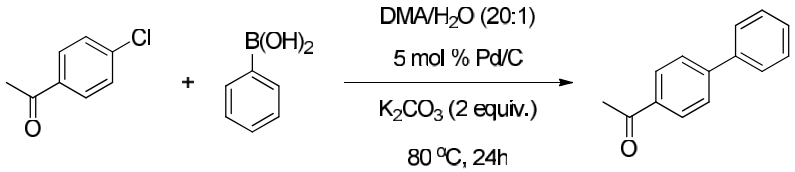
Reference: Miller, JA et al. *J. Org. Chem.* **1993**, *58*, 18-20.

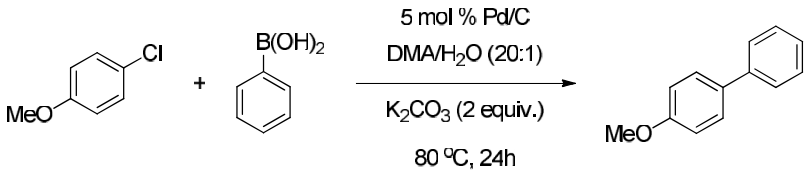
2. Diels-Alder reaction



Reference: Kraus, GA et al. *Green Chem*. **2011**, *13*, 2734-2736.

3. Suzuki reaction

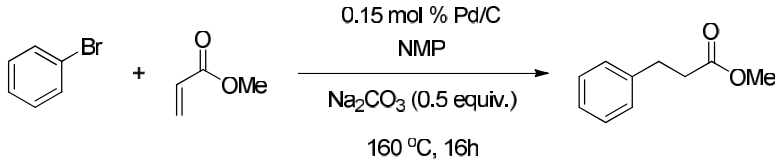




Reference: Sowa, Jr., JR et al. *Org. Lett.* **2001**, *3*, 1555-1557.

In general, the heterogeneous palladium from the Datye group allowed more of the desired product to form compared to the reaction using the commercial catalyst. The formation of the para-substituted benzoate was more selective with fewer side products in the presence of the novel palladium catalyst. The yields also increased from 51% to 60%, after switching catalysts. The Suzuki reaction was first run on a more favorable electron-deficient system, after which the experimentally prepared catalyst was used in the generally unreactive electron-rich system substituted with a chloride where the yields increased from 5% to 18%.

We are beginning trials with the Heck reaction, where a literature reference showed precedent for the coupled product via a heterogeneous catalyst.



Reference: Arai, M. et al. *React. Kinet. Catal. Lett.*, **2004**, *81*, 281-289.

Other Relevant Work

The Suzuki and Heck reactions described above are employed extensively in industry and academics. Improved catalysts for either of these transformations would have a significant impact. The alpha-olefin and Diels-Alder transformations depicted above are key transformations for Thrust 3 projects.

Plans for the Next Year

In the coming semester, the students will explore these reactions in greater depth and will further characterize the catalyst composition after the reactions.

Expected Milestones and Deliverables

The project will identify those industrially-significant reactions for which the catalysts developed by the Datye group are superior to commonly used homogeneous catalysts.

Member Company Benefits

Since the reactions to be studied are already of importance to many sectors of the chemical industry, the results will be of value to CBiRC industrial members.

Commercialization / Curriculum Impacts

We are still in the discovery phase of the project. Once new intellectual property is discovered the researchers will file disclosures.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title:ή 02-12F5 - Design of Carbon Nanocoated Oxides Supports without Mass Transfer Limitations for Production of α-Olefins from Carboxylic Acid

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Juan A. Lopez-Ruiz	**02/14/13**	**03/01/2012 to 02/28/2013**

ERC Team Members

Student Led Project Leader: Juan A. Lopez-Ruiz and Hien N. Pham

Supervising Faculty: Robert J. Davis at the University of Virginia and Abhaya Datye at the University of New Mexico

Research Assistant Professor: Hien N. Pham at the University of New Mexico

Students: Juan A. Lopez-Ruiz at the University of Virginia and Amanda Anderson at the University of New Mexico

Statement of Project Goals

Design and test a catalytic support without internal mass transfer limitations for the production of alpha olefins from carboxylic acids. Define the scope and limitations of the procedures.

Project's Role in Center's Strategic Plan

One of the integrative test beds in this Center involves the production of alkenes. Since fatty acids are readily produced by fermentation (Thrust 2), an efficient catalyst that converts fatty acids into alpha-olefins (plus CO and water) is needed.

Fundamental Barriers and Methodologies

The transformation of fatty acids into α-olefins has been studied very little compared to the conversion of fatty acids to saturated hydrocarbons. A major fraction of previous work in the area of deoxygenation of carboxylic acids uses dihydrogen, H_2, to prevent the catalyst from rapidly deactivating. Unfortunately, α-olefins are readily hydrogenated into paraffins in the presence of H_2 or isomerized in the presence of transition metal inside the catalyst pore. Furthermore, the catalyst deactivates really fast during reaction in gas phase conditions (573 K and 1 bar). Thus, the work in this project is to design a stable catalyst with high surface area and large pores to enhance the transport of the products out of the catalyst particle.

Achievements

We have successfully identified that metal sintering is the main cause of catalyst deactivation with electron microscopy and X-ray diffraction. Alcohol reduction (AR) and incipient wetness impregnation (IWI) were used to synthesize stable Pd nanoparticles on Norit and Vulcan carbon. Norit and Vulcan carbon show a Pd bimodal distribution after reaction. However, Norit carbon still contains small Pd nanoparticles, 2 – 3 nm, which could explain the higher activity than Vulcan carbon.

Liquid-phase reaction experiments were more selective towards the formation of isomers of 1-hexene, because of the rapid metal catalyzed isomerization. Gas-phase reaction experiments were more selective towards the formation of 1-hexene. However, the catalyst deactivation was more severe under gas-phase reaction conditions.

The attempts to design a stable catalyst with high surface area and macropores failed. The synthesized carbon nanocoated alumina supports were reactive under the reaction conditions (573 K, 37 bar, 0.01 cm^3min^{-1} of feed composed by 95 wt% heptanoic acid and 5 wt% dodecane). Calcination temperatures were changed from 673 to 1073 K to obtain a more graphitic and stable carbon nanolayer but the support was still reactive.

In summary, supported metal catalysts are being modified and characterized to understand the effects of support, and preparation conditions on the TOF, product selectivity, and catalyst stability in fatty acid decarbonylation.

Plans for the Next Term

We are working to optimize the catalyst synthesis preparation conditions to improve reaction rate and product selectivity towards the formation of α-olefins. We will synthesize silica based supports in order to understand the effect of pore size on catalyst activity and product selectivity.

Expected Milestones and Deliverables

The project will determine optimum conditions for catalyst synthesis to improve catalyst stability and activity.

Member Company Benefits

Members will have access to results from experimental studies of selective decarbonylation reactions catalyzed by supported metal catalysts. The member companies will also have access to catalyst synthesis and characterization techniques for supported metal catalysts.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **02-13S1** - Rapid, High Throughput Identification and Quantification of Carbohydrates and Their Derivatives Using UPLC-PDA-ELSD

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*: **02/28/2013**	Reporting Period: **03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Jean-Philippe Tessonnier, Iowa State University

Other Investigators: Laura Jarboe and Adam Okerlund, Iowa State University

Graduate Students: Alex Liu and Tao Jin, Iowa State University

Undergraduate Student: Kyle Weis, Iowa State University

Statement of Project Goals

Carbohydrates produced from cellulose will play an increasing role in the production of biorenewable chemicals. For example, significant efforts are dedicated to the conversion of cellulose-derived glucose to furanics such as 5-hydroxymethylfurfural (HMF). However, the identification and quantification of carbohydrates remain challenging. It has traditionally been done using either gas chromatography (GC), which requires additional steps of derivatization to convert sugars into volatile compounds, or high pressure liquid chromatography (HPLC) with a refractive index (RI) detector. Both techniques suffer various limitations which all negatively impact the throughput of the analytical equipment and the accuracy of the quantification. Ruiz-Matute et al. reviewed the various derivatization techniques to analyze and quantify carbohydrates by GC [1]. Although these techniques all improve the volatility of the carbohydrates and make the analysis possible, they are complex, time consuming, present variable yields, the obtained compounds are often moisture sensitive, and they lead to more than one peak when tautometers can form [1]. For HPLC, the physical characteristics of the RI detector require an isocratic elution, which significantly prolong the analyte's retention time and induce peak broadening, thus reducing the accuracy of the quantification [2, 3].

The Tessonnier group recently purchased an ultra-high pressure liquid chromatography (UPLC) instrument equipped with both photodiode array (PDA) and evaporative light scattering detectors (ELSD). This state of the art instrument solves the drawbacks of HPLC by combining pressures up to 12,000 psi with a new detection technology compatible with gradient elution. The preliminary tests we performed for common sugars showed that the run time drastically decreased from 1 hour with HPLC to less than 10 minutes, and at the same time, allowed a better separation and resolution of the peaks. In the proposed project, we will develop a rapid UPLC-PDA-ELSD method, which will enable the identification and quantification of both sugars and furanic compounds using a single instrument configuration. Preliminary results showed that the separation of levoglucosan, glucose, and fructose can be achieved in less than 2 minutes, as well as the separation of HMF and

furfural in less than 1 minute.

We plan on developing this method as a collaborative effort between the Tessonnier group (Thrust 3), the Jarboe group (Thrust 2), and the Okerlund group (Translational Research). Each group will develop methods for compounds of interest for their research, and these methods will then be combined to produce a comprehensive, rapid, high throughput UPLC method. We believe that this project will lay the foundation for new interactions between the three groups, and more generally, across disciplines within CBiRC. The developed method can potentially be used by any team within CBiRC working on the conversion of carbohydrates to organic compounds using either chemical or biological catalysts. Results will be compared with existing HPLC methods [2, 3] to demonstrate the improvements, and a manuscript will be submitted to a peer-reviewed journal.

Foreign Collaborations

Not applicable.

Achievements

This project was only recently funded through the Center's Student-led Research Grant program. Consequently, there are no achievements to report at this time. The work is not scheduled to commence until 3/1/2013, after the annual report has been submitted.

Expected Milestones and Deliverables

We expect to deliver a rapid UPLC-PDA-ELSD method to identify and quantify carbohydrates such as glucose, fructose, levoglucosan as well as furanic compounds such as HMF and EMF. A final project report will be submitted at the end of the six-month period, and we will give a presentation during CBiRC's annual meeting.

Technology Impacts

The resulting method will be shared between all groups, and a short communication will be published in a peer-reviewed journal.

References

[1] A.I. Ruiz-Matute, O. Hernández-Hernández, S. Rodriguez-Sánchez, M.L. Sanz, I. Martinez-Castro, Derivatization of carbohydrates for GC and GC-MS analyses, Journal of Chromatography B, Volume 879, Issues 17-18, 15 May 2011, pp. 1226-1240, ISSN 1570-0232, 10.1016/j.jchromb.2010.11.013. (http://www.sciencedirect.com/science/article/pii/S1570023210007063)

[2] Xuejun Liu, Ning Ai, Haiyan Zhang, Meizhen Lu, Dengxiang Ji, Fengwen Yu, Jianbing Ji, Quantification of glucose, xylose, arabinose, furfural, and HMF in corncob hydrolysate by HPLC-PDA-ELSD, Carbohydrate Research, Volume 353, 15 May 2012, pp. 111-114, ISSN 0008-6215, 10.1016/j.carres.2012.03.029. (http://www.sciencedirect.com/science/article/pii/S0008621512001371)

[3] Rui Xie, Maobing Tu, Yonnie Wu, Sushil Adhikari, Improvement in HPLC separation of acetic acid and levulinic acid in the profiling of biomass hydrolysate, Bioresource Technology, Volume 102, Issue 7, April 2011, pp. 4938-4942, ISSN 0960-8524, 10.1016/j.biortech.2011.01.050. (http://www.sciencedirect.com/science/article/pii/S0960852411001192)

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **33-12F3 IAB Seed Project -** High Throughput Facility for Reaction Kinetics Measurements in the Development of Amino-Acid Tolerant Heterogeneous Catalysts

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
James Dumesic	**02/14/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: James Dumesic, University of Wisconsin-Madison

Faculty: Abhaya K. Datye, University of New Mexico; Nancy DaSilva, University of California - Irvine

Graduate Students and Staff: Thomas Schwartz, University of Wisconsin-Madison

Statement of Project Goals

The goal of this project is the development of a high throughput facility for reaction kinetics measurements which can be used to efficiently characterize the effects of biogenic impurities on catalysts produced within CBiRC.

Project's Role in Center's Strategic Plan

The presence of small amounts of biogenic impurities is a critical factor in the upgrading of biologically-synthesized materials, as even small amounts of compounds such as amino acids can be detrimental to catalytic activity. However, these effects are not necessarily well documented, and the facility under development will fill this void in the literature. Such work is in line with the Center's strategic interests in upgrading of biologically-derived compounds, such as pyrones, for which we have observed that catalyst inhibition is a significant barrier to commercially viable production of specialty chemicals.

Fundamental Barriers and Methodologies

Of these barriers, one of the most significant is the depression of activity of heterogeneous catalysts by the action of biogenic impurities. Previous work in CBiRC, highlighted in project T3.9, has demonstrated that amino acids, among other biogenic compounds, are inhibitory to palladium hydrogenation catalysts for pyrone conversions. In this regard, with money from CBiRC's Industrial Advisory Board, we have begun to develop a high throughput facility for measurement of reaction kinetics in the presence of a broad range of biogenic impurities and for an array of standardized reactions, thereby providing a means for comparing these effects on a consistent basis.

Achievements

This high-throughput screening facility will enable rapid evaluation of the efficacy of catalysts

for chemical transformations under varied reaction conditions. Specifically, members will be able to effectively identify biogenic compounds (e.g., amino acids, vitamins) responsible for catalytic deactivation, and conversely, catalysts resistant to these biogenic compounds. In this manner, identification of cell culture conditions and heterogeneous catalysts amenable to process integration can be performed rapidly. This identification will be made based on a series of well-defined probe reactions, the choice of which depends on whether the catalyst is an acid or metal. For hydrogenation catalysts, probe reactions will focus on hydrogenation of specific functional groups such as C=C bonds and C=O bonds. Acid catalysts will be evaluated based on dehydration, esterification, and alkane isomerization.

As researchers in Thrust 3 develop new catalysts, the tolerance for these towards biogenic impurities will be evaluated on a consistent basis. These catalysts may be designed specifically to achieve high stability in the presence of inhibitors, in which case evaluation in the context of well-characterized probe reactions will provide a quantitative measure of improvement over base-case catalysts. Conversely, labs may develop new catalysts designed to target specific reactions in existing or future testbeds, and these can be screened early on for potential deactivation in the presence of contaminants typical of the chosen feedstock.

The utility of the high-throughput screening facility will not be limited to testing novel catalysts. Different types of culture media provided by labs in Thrust 2 can be screened using an equivalent, base-case catalyst and the above-mentioned probe reactions. Information collected by this screening will amount to a standardized database indicating the effects of various cell culture media on heterogeneous catalysts. This will be useful in two ways. Primarily, the database can be referenced by Thrust 3 at the outset of a project or the startup of a new testbed; the influence of the culture medium can be taken into account even while choosing a catalyst to perform a particular reaction in the desired pathway. Additionally, members of Thrust 2 can use the database to provide guidance when scaling-up cell culture so as to minimize the requirements for downstream separations. Yet another advantage of the facility will be the ability to screen many inhibitors under equivalent conditions simultaneously (or a single inhibitor under many conditions), thus providing an efficient way to standardize the impact of potential inhibitors pinpointed by all three Thrusts.

Other Relevant Work

The successful integration of chemical and biocatalysis requires the development of a knowledge base that is, at present, only scarcely represented in the chemical or bioengineering literature. There are barriers to successfully combining these two historically independent areas which CBiRC is, by the nature of its inception, one of the first to identify. There is scant literature about this effect, although it has been shown that amino acids[1] and sulfur-containing degradation products of proteins or isothiocyanates from glucosynolates[2] have a measurable impact of the hydrogenation activity of metal catalysts. Eventual publication of the results obtained by this facility will help to fill this void in the engineering knowledge base.

Plans for the Next Five Years

After initial equipment purchasing and commissioning (phase 1, in progress), operational protocols will be developed (phase 2) which will examine the specific impacts of targeted biogenic impurities identified by members of Thrusts 1 and 2 as likely to be found in culture media. Each phase is

anticipated to take six months. Following this one year start-up period, the facility will be made available for use by the members of CBiRC at large.

Expected Milestones and Deliverables

Purchase of equipment is imminent, and commissioning will take place shortly thereafter. Following this, operational protocols for the facility will be established. The facility will then be ready for use by members. As data is collected and developed into a database, it will be made available to the CBiRC community.

Member Company Benefits

Members will have access to unpublished results from experimental studies.

Commercialization / Technology Transfer

References

1. Zhang, Z.; Jackson, J. E.; Miller, D. J., Effect of biogenic fermentation impurities on lactic acid hydrogenation to propylene glycol. Bioresour. Technol. 2008, 99 (13), 5873-5880.
2. Brands, D. S.; U-A-Sai, G.; Poels, E. K.; Bliek, A., Sulfur Deactivation of Fatty Ester Hydrogenolysis Catalysts. J. Catal. 1999, 186, 169-180.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: ERC - Small Business: Commercialization of Furanic-Based Biorenewable Chemicals

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adam Okerlund	**02/28/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Dr. Adam Okerlund, Iowa State University

Undergraduate Students: Natalia Rodriguez Quiroz, Kossi Sessou, and Kyle Weis, Iowa State University

Other Staff: Brendan Babcock, Elliot Combs, and Dalton Hughes, Iowa State University

Industrial Participants: Glucan Biorenewables, LLC.

Statement of Project Goals

5-hydroxymethylfurfural (HMF) is a potentially valuable platform chemical that currently cannot be made cost-effectively. We have identified a cost-effective method for the selective dehydration of glucose and starch to produce HMF. This same technology can also be utilized for the formation of other furanic molecules. The goal of this project is to **explore the techno-commercial potential** surrounding the production and downstream processing conditions for HMF and other furanics using technology developed within CBiRC. The cost-effective production of the furanics will open the path to making furan-based building blocks used in production of plastics, fine chemicals, diesel fuel and fuel additives.

Project's Role in Center's Strategic Plan

CBiRC is striving to create an innovation ecosystem that supports the development of new technologies that could transform the petroleum-based chemical industry to a bio-based industry. Such emerging technologies often carry considerable techno-commercial risk and hence become opportunities for an early-stage company. The funding of this project and others has created an opportunity to found Glucan Biorenewables, a new startup entity. The project acts as a first example for CBiRC's innovation ecosystem through the formation of a translational research project supporting a new startup.

Fundamental Barriers and Methodologies

HMF is an intermediate step in the decomposition pathway of glucose to levulinic acid. Previous reports have shown enhanced glucose conversion and HMF selectivity in acidic aqueous systems combined with salts, particularly chloride salts such as MgCl2 and AlCl3, the application of pressure, and biphasic extraction. These promising results were the basis for the "ERC - Small Business: Commercialization of Furanic-Based Biorenewable Chemicals" grant.

A new method for the selective dehydration of glucose, xylose, and polysaccharides to produce furan derivatives such as HMF and furfural has been established. This new method appears more commercially viable than those previously developed because it can use polysaccharides to yield a high concentration of HMF and furfural. CBiRC is collaborating in this effort with a small business partner, Glucan Biorenewables, which has a licensing agreement with the University of Wisconsin. This effort will modify the fundamental research at the University of Wisconsin to take into account scale-up and ultimate production of furanics. The technology developed in partnership with Glucan Biorenewables will translate into the production of HMF and other furanics where a larger opportunity may exist.

Foreign Collaborations

None.

Achievements

The first year of this project had focused on probing existing information to advance the understanding of the biphasic reactor system and how it could become amenable to industrial scale HMF production. After reviewing current information, the decision was made to focus on the water tolerant lanthanide series as the acid catalyst. These rare earth elements serve as Lewis acid catalysts and are viable without pH adjustment. The ability to perform the glucose dehydration reaction at a neutral pH eliminates the costly specialized equipment necessary at scale for highly acidic environments. Other reaction condition variables that were investigated were the solvent used as the extracting phase in the biphasic system and the salt used to saturate the aqueous phase. Using a lower boiling point extraction solvent allowed for its potential removal away from HMF via distillation. The high salt content in the biphasic reaction is not ideal, but plays a crucial role in partitioning. Progress was made in regards to integrating the biphasic reaction and purification efforts, but these efforts were put on hold when a novel reaction system was developed in the Jim Dumesic laboratory at the University of Wisconsin.

Glucan Biorenewables has subsequently taken an option to license this novel reaction system technology. Experimentation moving forward from Glucan Biorenewables taking the option to license has focused on the integration of the novel reaction technology and a separation scheme to purify furanic molecules. Furanic molecules under consideration for production include, but are not limited to, HMF, furfural, 2,5-furandicarboxylic acid (FDCA), and levulinic acid. Market indicators and investment partners will have just as much of a role in shaping the future experimentation as the development of the basic fundamentals needed to integrate the reaction and separation technologies.

Other Relevant Work

The production of HMF and other furanics from biorenewable feedstocks has become a hot topic over the last decade. Some groups have focused on using fructose as a feedstock for six carbon furanic molecules due to higher yields while others work with glucose and polysaccharide feedstocks. The reaction systems for the saccharide conversion to furanics vary and include ionic liquids, supercritical water, biphasic liquid-liquid, and acidic aqueous conditions.

The James Dumesic (University of Wisconsin-Madison) and Brent Shanks (Iowa State University) groups within CBiRC are working on related saccharide dehydration projects.

Plans for the Next Year

The technical plans for the next year revolve around the sustained development of a continuous reaction system and the subsequent downstream processing. Glucan Biorenewables needs to establish a suitable biomass feedstock which will allow for cost-effective production of the desired furanic compounds at industrial scale. This will largely be accomplished through reaction studies using the semi-continuous reaction system. An optimum solvent for the production as well as purification of the desired furanic compounds will also need to be selected. The reaction and purification studies will occur in parallel with information from each being shared to better optimize the entire process. Once a completed process is in place, a techno-commercial analysis will be performed to highlight potential pitfalls in a scaled-up process.

Expected Milestones and Deliverables

- March 2013 – June 2013: Develop and demonstrate saccharide to furanic conversion and separation capabilities in a continuous flow system
- March 2013 – August 2013: Perform a techno-commercial analysis to highlight potential pitfalls in a scaled-up system

Member Company Benefits

This project is connected through its funding to Glucan Biorenewables, one of the member companies working closely with CBiRC. This sponsored translational research project allows the startup entity to further evaluate the scale-up feasibility for the developing technology.

Commercialization / Curriculum Impacts

Glucan Biorenewables has taken an option to license the base technology at the core of this project. Glucan Biorenewables has also formed a strategic commercialization partnership with an outside entity. The new partnership brings together the business capability of the outside entity with the technical knowhow of Glucan Biorenewables to facilitate marketing and re-risking of the furanics based project. Funding for commercialization is attempting to be procured from venture capital entities, downstream industries interested in furanics, as well as through government programs such as SBIR.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Grow Iowa Values Fund: Catalytic Conversion Platform for Furan Derivatives

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adam Okerlund	**02/28/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Dr. Peter Keeling, Iowa State University

Other Faculty: Dr. Brent Shanks, Iowa State University; Dr. James Dumesic, University of Wisconsin, Madison

Undergraduate Students: Natalia Rodriguez Quiroz, Kossi Sessou, and Kyle Weis, Iowa State University

Other Staff: Dr. Adam Okerlund, Iowa State University

Industrial Participants: Focus First L.P. in partnership with Glucan Biorenewables, LLC

Statement of Project Goals

Research and development at CBiRC has identified a cost-effective method for the selective dehydration of mono and polysaccharides to produce furanic molecules. We must now implement a cost-effective and scalable downstream separation and purification strategy for these molecules. The goal of this project is the research and development of the cost-effective **purification strategy** with the ultimate goal of obtaining high purity furanics.

Project's Role in Center's Strategic Plan

CBiRC is striving to create an innovation ecosystem that supports the development of new technologies that could transform the petroleum-based chemical industry to a bio-based industry. Such emerging technologies often carry considerable techno-commercial risk and hence become opportunities for an early-stage company. The funding of this project and others has created an opportunity for Glucan Biorenewables, a new startup entity, to continue advancing furanic production technology. Focus First is interested in realizing the potential of Glucan Biorenewables through highly focused investments designed to develop a commercializable product. The project acts as an example of CBiRC's innovation ecosystem through the formation of a translational research project supporting a new startup.

Fundamental Barriers and Methodologies

A new proprietary method for the selective dehydration of glucose, xylose, and polysaccharides to produce furan derivatives such as HMF and furfural has been established. This new method appears more commercially viable than those previously developed because it can use polysaccharides to yield a high concentration of HMF and furfural. These promising results were the basis for the Grow Iowa Values Fund - Catalytic Conversion Platform for Furan Derivatives

grant. While the reaction of saccharides to furanics has been broadly researched and developed, the purification of the furanic molecules from the proprietary reaction system has not.

CBiRC is collaborating in this effort with a small business partner, Glucan Biorenewables, which has a licensing agreement with the University of Wisconsin for the proprietary reaction system. The separations research in this project will modify the fundamental research at Glucan Biorenewables to take into account scale-up and ultimate production of furanics. The methodology of this project focused on finding a best-case purification scheme for furanic molecules based on capital investments, consumable expenses, and energy costs to build a business case for commercialization.

Foreign Collaborations

None

Achievements

Information and know-how for the cost-effective furanic production was obtained from other projects within CBiRC as well as proprietary research within Glucan Biorenewables. Most of the production methods employ a proprietary system where mono or polysaccharides convert to either five or six carbon furan derivatives. We have now focused our efforts on the extraction of the furan derivatives. Care has been taken to only research strategies that are potentially feasible at an industrial production scale. After reviewing pertinent literature and examining the chemical properties of the reaction constituents, the decision was made to focus on the three means of purification; liquid-liquid extraction, adsorption onto a solid phase, and distillation. The mode of purification researched is dependent on the desired furan derivative and the choice of solvent.

Most separation research to date for this project has utilized distillation. The high boiling points of several furanic molecules, the presence of humins, and the reactivity of furanic molecules at high temperature combine to make this a difficult purification approach. However, recent distillation results have shown promise.

Other Relevant Work

The production of HMF and other furanics from biorenewable feedstocks has become heavily researched over the last decade, but little advancement in a purification strategy has been developed. Partial to full purification of some furan derivatives has been achieved by other groups by using adsorption onto acidic ion exchange or non-functional polymeric resins, binding to soluble polyethyleneimine, and distillation. Previous purification research of furanic molecules had been performed within CBiRC under the project "Grow Iowa Values Fund: Furanics-Based Biorenewable Chemicals". The techno-economic potential of furanic molecules is being analyzed within CBiRC under the project "ERC - Small Business: Commercialization of Furanic-Based Biorenewable Chemicals".

Plans for the Next Year

Funding for this sponsored project ends May 31, 2013. We will continue purification efforts up until that date to help Glucan Biorenewables build a business case to secure funding for commercialization.

Expected Milestones and Deliverables

March 2013 – May 2013: Establish a distillation separations scheme for furanic molecules of interest.

Member Company Benefits

This project is connected through its funding to Glucan Biorenewables, one of the member companies working closely with CBiRC. This sponsored translational research project allows the startup entity to further evaluate the scale-up feasibility for the developing technology.

Commercialization / Curriculum Impacts

Glucan Biorenewables has taken an option to license to the base technology at the core of this project and has been able to secure a commercialization partner with an outside entity.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Grow Iowa Values Fund: Furanics-Based Biorenewable Chemicals

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adam Okerlund	**02/28/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Dr. Adam Okerlund, Iowa State University

Undergraduate Student: Dalton Hughes, Iowa State University

Other Staff: Brendan Babcock, Iowa State University; Elliot Combs, Iowa State University

Industrial Participants: Glucan Biorenewables, LLC.

Statement of Project Goals

Research and development at CBiRC has identified a cost-effective method for the selective dehydration of glucose and starch to produce HMF. We must now implement a cost-effective and scalable downstream separation and purification strategy for HMF. The goal of this project is the research and development of the cost-effective **purification strategy** with the ultimate goal of obtaining high purity HMF.

Project's Role in Center's Strategic Plan

CBiRC is striving to create an innovation ecosystem that supports the development of new technologies that could transform the petroleum-based chemical industry to a bio-based industry. Such emerging technologies often carry considerable techno-commercial risk and hence become opportunities for an early-stage company. This project was funded under the NSF 10-617 solicitation and the Grow Iowa Values Fund, creating an opportunity to found Glucan Biorenewables, a new startup entity. The project acts as a first example for CBiRC's innovation ecosystem through the formation of a translational research project supporting a new startup.

Fundamental Barriers and Methodologies

HMF is an intermediate step in the decomposition pathway of glucose to levulinic acid. Previous reports had shown enhanced glucose conversion and HMF selectivity in acidic aqueous systems combined with salts, particularly chloride salts such as MgCl2 and AlCl3, the application of pressure, and biphasic extraction. These promising results were the basis for the Grow Iowa Values Fund - Furanics-Based Biorenewable Chemicals grant. While the biphasic reaction of saccharides to HMF had been broadly researched and developed, the purification of HMF from an organic extraction phase had not.

Information and know-how for the cost-effective HMF production was obtained from other Thrust 3 projects within CBiRC. Most of the production methods employ a biphasic system where HMF

preferentially resides in the organic phase. We must now extract the HMF from this organic phase in high yields and at low cost. The methodology of this project focused on finding a best-case (least-cost) purification scheme for HMF from the organic extraction phase based on capital investments, consumable expenses, and energy costs to build a business case for commercialization.

Foreign Collaborations

None

Achievements

This project focused on researching several purification strategies of HMF from the organic phase of the biphasic reactor system. Care was taken to only research strategies that were potentially feasible at an industrial production scale. After reviewing pertinent literature and examining the chemical properties of the HMF versus the organic extraction phase, humins, and other contaminants, the decision was made to focus on the three means of purification; liquid-liquid extraction, adsorption onto a solid phase, and distillation.

The most successful means of purification attempted for HMF was by liquid-liquid extraction with water from the post-reaction organic extraction phase. HMF has high solubility in pure water. The salt used during the glucose to HMF dehydration reaction not only helps to facilitate the separation of the phases, but also helps to lower the solubility of HMF in the aqueous phase. After the reaction is completed, the salt saturated aqueous phase is removed and replaced with fresh water. The ability to perform this type of purification is dependent on the polarity of the extraction phase. If the organic extraction phase is too polar, it becomes miscible with water in the absence of salt eliminating liquid-liquid extraction possibilities. Additives to the aqueous and organic phases have also shown promise to facilitate a biphasic separation of HMF in the absence of salt.

The adsorption of HMF onto solid phase resins and the distillation of HMF both showed potential, but would require solvent changes detrimental to the glucose to HMF yield. A variety of solid phase resins were employed to bind either HMF or a portion of the contaminants. A scheme was devised for purification on the silica resins, but this scheme must still be developed before it can become a practical process at scale. The distillation of HMF from the extraction solvent was another means of separation attempted. The high boiling points of HMF and the extraction solvent, the presence of humins, and the reactivity of HMF at high temperatures combined to make this a poor purification strategy. Attempts to use a lower boiling point extraction solvent resulted in lower liquid-liquid extraction yields.

The purification efforts performed in the project helped Glucan Biorenewables build a business case to secure a commercialization partner.

Other Relevant Work

The production of HMF from biorenewable feedstocks has become heavily researched over the last decade, but little advancement in a purification strategy has been developed. Partial purification has been achieved by other groups by using adsorption onto acidic ion exchange or non-functional polymeric resins, binding to soluble polyethyleneimine, and distillation. Further research in the purification of furanic molecules is being performed within CBiRC under the project “Grow Iowa

Values Fund: Catalytic Conversion Platform for Furan Derivatives".

Plans for the Next Year

This project ended May 31, 2012. No work is planned for the next year.

Expected Milestones and Deliverables

May 31, 2012: Project end date. Work completed up to the project endpoint will be incorporated into current and future furanics-based research.

Member Company Benefits

This project is connected through its funding to Glucan Biorenewables, one of the member companies working closely with CBiRC. This sponsored translational research project allows the startup entity to further evaluate the scale-up feasibility for the developing technology.

Commercialization / Curriculum Impacts

Glucan Biorenewables has taken an option to license to the base technology at the core of this project and has been able to secure a commercialization partner with an outside entity.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Proof of Concept Initiative: Biobased Production of Terephthalic Acid

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
George Kraus	**02/18/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Dr. George A. Kraus, Iowa State University

Other Faculty: Dr. Brent Shanks, Iowa State University

Graduate Students: Gerry Pollock, Iowa State University

Undergraduate Students: Natalia Rodriguez-Quiroz, Kossi Sessou and Kyle Weis, Iowa State University

Other Personnel: Drs. Peter Keeling and Adam Okerlund, Iowa State University

Statement of Project Goals

The project goals are to:

1. Identify a scalable process for producing coumalic acid from malic acid.
2. Improve the conversion of coumalic acid to para-toluic acid.

Project's Role in Center's Strategic Plan

Malic acid can be produced from fermentation of glucose in high titer. This project uses malic acid and employs chemical catalysis to develop a route to terephthalic acid, a commodity chemical that is currently derived solely from petroleum feedstocks.

Fundamental Barriers and Methodologies

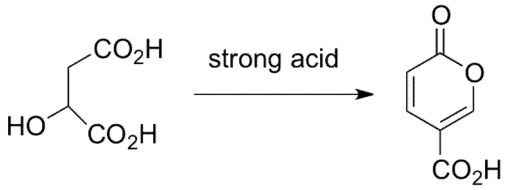
The terephthalic acid process developed at Iowa State involves converting malic acid, available via either biochemical or chemical catalysis routes, into coumalic acid. The coumalic acid undergoes a one-pot cycloaddition/elimination/oxidation to generate para-toluic acid. Para-toluic acid has been transformed into terephthalic acid in one step. Reaction optimization and scale up of both reactions beyond the laboratory scale will be necessary in order to enable this process to gain industry adoption.

Foreign Collaborations

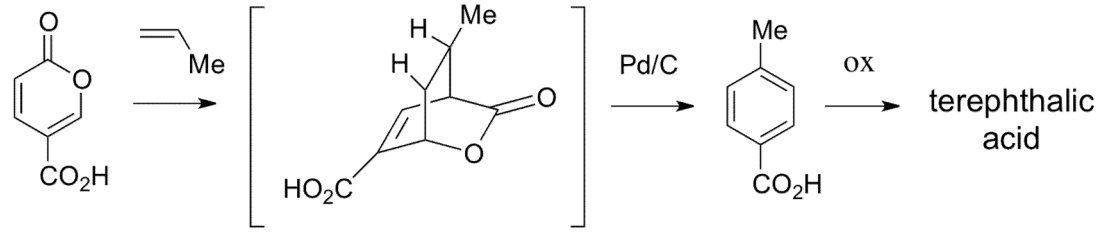
None

Achievements

The Kraus group has discovered what appears to be a very efficient process using chemical catalysis to convert commercially available malic acid into coumalic acid. The chemical process utilizes an acid catalyst in an inexpensive organic solvent at temperatures ranging from 80 °C to 100 °C. This process produces crystalline coumalic acid in 85% yield on a ten-gram scale. This process has the potential to replace the only existing process, which uses sulfur trioxide in a sulfuric acid solvent. Invention disclosures have been filed with the ISU Research Foundation. A provisional patent needs to be filed.



With regard to the second goal, improving the conversion of coumalic acid to para-toluic acid, the Kraus and Okerlund groups have characterized the commercial palladium catalyst used in the laboratory scale experiments. They have decreased the amount of catalyst needed by 75% and have determined an optimal temperature and solvent. Catalyst studies involved the evaluation of 5% nickel on carbon catalysts, 5% ruthenium on carbon catalysts, 5% platinum on carbon catalysts, as well as 2%, 5% and 10% palladium on carbon catalysts. The reaction temperature was varied from 100 °C to 225 °C.



Other Relevant Work

Ultimately, the aim is to make coumalic acid into a platform molecule with a range of chemical outcomes. The Kraus group has shown that coumalic acid and methyl coumalate react with alpha-olefins, electron-deficient alkenes, and electron-rich alkenes to generate aromatic carboxylic acids in good to excellent yields. CBiRC researchers are applying for NSF funding from the PFI-AIR "Research Alliance" solicitation.

Plans for the Next Year

Two reaction parameters to be studied are the influence of the nature of the catalyst support and the optimal ratio of propene to coumalic acid. We will evaluate the conversion of malic acid to coumalic acid using heterogeneous acid catalysts.

Expected Milestones and Deliverables

With proof of feasibility already in place for malic acid production, there remains the need to develop the proof of concept in place for both the coumalic acid production and catalysis to biobased chemicals. A critical aspect of the work will be perfecting the catalysis steps using malic acid actually produced by fermentation, which may contain impurities that might affect the transformation. This will lead to a deep understanding of reaction kinetics for the conversion steps and a detailed process model.

Member Company Benefits

Terephthalic acid is a compound of interest to a number of CBiRC companies. Identifying a biobased route to terephthalic acid has become a major objective. This research will demonstrate the viability of our pathway to terephthalic acid.

Commercialization / Curriculum Impacts

We have submitted two disclosures to the ISU Research Foundation related to the terephthalic acid pathway. We plan to meet with them in March 2013.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Selective Dehydration of Multifunctional Substrates

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Brent Shanks	**02/13/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Brent Shanks, Iowa State University

Collaborator: George Kraus, Iowa State University

Graduate Students: Michael Nolan, Uma Wanninayake, and Anita Bejile, Iowa State University

Undergraduate Students: Geng Sun, Iowa State University

Collaborating Member: USDA NCAUR

Statement of Project Goals

Dehydration is an important reaction for removing oxygen present in biobased feedstocks. Mono-alcohol dehydration has been studied extensively, but hydroxyl removal in the presence of other functional groups has received considerably less attention. Polyhydroxylated molecules are commonly encountered when trying to convert carbohydrate and carbohydrate-derived species to more deoxygenated molecules. Several fundamental questions arise when evaluating the dehydration of polyols. First, if multiple hydroxyl groups are present, which one is most reactive? What is the relative reactivity of hydroxyl groups on consecutive carbons versus those not on consecutive carbons? Dehydration of polyols can lead to unsaturated alcohols and/or cyclic ethers. Can the relative selectivity to these product compounds be modified by catalyst choice? To create a catalytic "tool chest" for biorenewable chemicals there is a need to determine the catalyst design rules required to selectively manipulate the removal of oxygen via dehydration.

Project's Role in Center's Strategic Plan

The ability to perform selective dehydration of a multifunctional substrate is a key capability in the catalytic conversion of biobased molecules. This project will contribute to establishing reaction rules for these dehydration reactions with the goal of developing a catalytic "tool box."

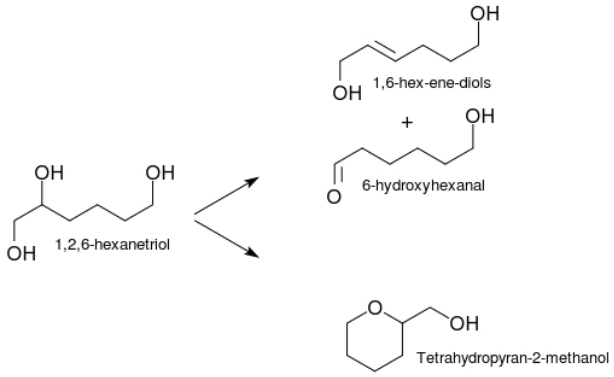
Fundamental Barriers and Methodologies

An assimiliation of the dehydration literature on polyhdroxylated reactants suggests that acid catalyzed dehydration of polyhydroxylated reactants generally shows two major selectivity rules. First, diol pairs that are (n,n+3) to (n,n+4) will readily form into the corresponding furan or pyran ring under most reaction conditions, and require an acid-metal bifunctional catalyst to ring-open. Second, when the diol pair is closer to each other, an elimination of a hydroxyl group occurs, leaving behind a double bond on the dehydrated polyol. If the double bond forms adjacent to another alcohol, i.e., an enol, the molecule will readily intraconvert to the

corresponding ketone or aldehyde. While these reaction trends have been established for diols, they have not been extended to more hydroxylated compounds. Additionally, there is a need to determine to what extent the selectivity to different products can be modified by catalysts that have both acidic and metallic functions.

Achievements

This project has just been initiated, so we have limited results. Previous work suggested in the dehydration of 1,2,6-hexanetriol that linear products proceeded through an epoxide intermediate. The proposed epoxide was synthesized and found to convert uniquely to the linear products. We also found that once formed the ring product would not convert to the linear products. Therefore, we currently think the two reaction paths shown in the figure to the right are in fact in competition.



Other Relevant Work

See the Selective Dehydration project (T3.2) summary.

Plans for the Next Year

There is overlap between this sponsored project and ongoing work with CBiRC core funding, so this additional funding will be used to expand the polyhydroxylated molecules we are exploring in the dehydration studies. We also plan to work with NCAUR to synthesis reaction intermediate products to help understand the mechanisms of selective dehydration as a function of catalyst type.

Expected Milestones and Deliverables

1. Develop an understanding of the catalyst characteristics that correlate to the linear/ring ratio in the dehydration of 1,2,6-hexanetriol.
2. Develop a mechanistic model for the 1,2,6-hexanetriol dehydration reaction to determine the key step(s) that dictate this selectivity.
3. Extend the dehydration experiments to other polyhydroxylated reactants.
4. Develop an overarching framework for how polyhydroxylated reactants dehydrate as a function of key catalyst characteristics.

Member Company Benefits

To guide the selection of attractive polyhydroxylated intermediate molecules there is a need to understand how these types of molecules dehydrate.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **LCA -** Techno-Economic Analysis of Making Hydrocarbons from Biomass-Derived Sugars

Thrust: Research Support - Life Cycle Assessment (LCA)

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Robert Anex	**03/04/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Robert Anex (University of Wisconsin), Sampath Gunukula (University of Wisconsin), Raj Raman (Iowa State University), Joshua Claypool (Iowa State University)

Statement of Project Goals

The objective of this study is to evaluate the techno-economic feasibility of hydrocarbons produced from low-cost biomass-derived sugars with particular emphasis on evaluating and informing the development of our testbeds. The testbed technologies are in early development so only preliminary experimental information is available that informs the likely operating conditions of the major unit processes. However, it is precisely at this early stage of development that we need to identify the major bottlenecks and research priorities that will make these processes economically feasible and direct our research efforts accordingly. We have developed methods for early-process 'bounding analyses' that allow us to screen technology pathways. We have three main activities in this project: 1) developing methods for early screening of process economic and technical feasibility; 2) developing detailed models and databases of processes early in the life-cycle that are common to all chemicals and will be needed for detailed analysis as technology pathways are more fully defined; 3) evaluation of alternative routes to our target chemical platforms.

Project's Role in Center's Strategic Plan

This project is central to achieving the Center's strategic objectives. The Life Cycle Assessment (LCA) support area includes development and application of a variety of assessment methods that guide the research and development direction of the individual thrusts and the Center's overall priorities. Analysis of the techno-economic feasibility and environmental impact of proposed technology pathways from biorenewable resources to chemicals will identify technology bottlenecks and environmental constraints that must be addressed through research or system reconfiguration. The LCA activities in evaluating the testbeds also provides vehicle to integrate the thrusts and individual projects of the center. Understanding the trade-offs that are inherent in choices made within the thrusts requires an undestanding of the full center mission and technology life cycle. Thus the LCA analyses are carried out in close collaboration with the engineers and scientists working in the thrusts and foments a deep and meaningful discussion among the thrusts leading to a truly transdisciplinary understanding of and approach to the Center mission.

Fundamental Barriers and Methodologies

One fundamental question for this Center is how to predict during the earliest stages of development of a chemical pathway its technical, economic and environmental feasibility. The Center has the capacity to develop thousands or perhaps tens of thousands of pathways, but we must screen these at the earliest stages of development and select only the most promising for development. One dimension of this screening requires techno-economic and life-cycle assessment methods that can be discriminate between pathways.

Another fundamental question for CBiRC is: at what point chemicals developed by the biological catalyst platform should be handed off to the chemical catalyst platform. A first step to understanding this is to develop models of the technical and economic dimensions of converting the intermediates via chemical catalyst to valuable end products (e.g., alkanes) so that different intermediate starting points can be evaluated.

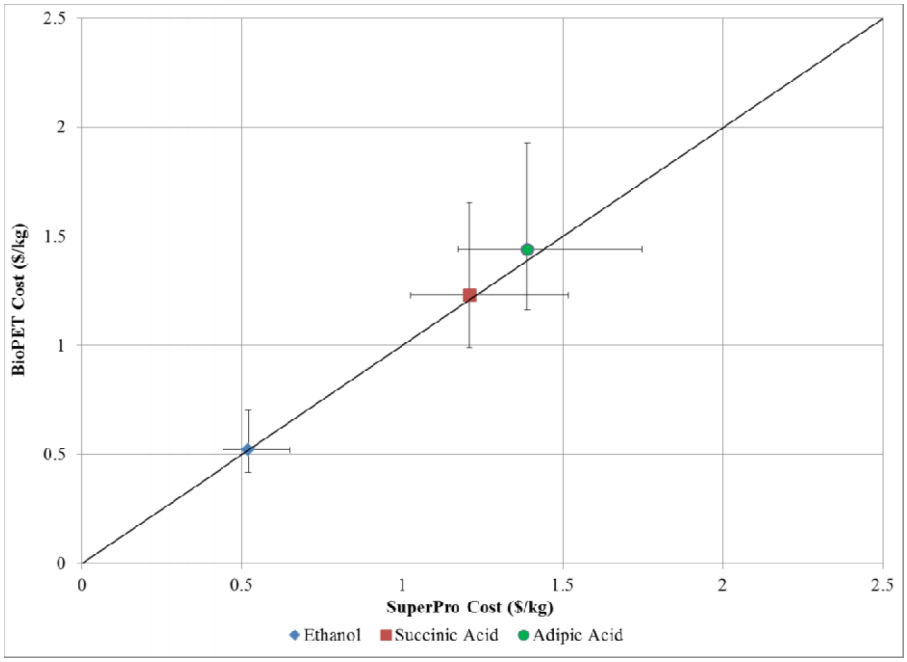
Achievements

We have developed a method for screening and framing the economic feasibility of biorenewable chemicals at very early stages of development. We have applied this method to the two testbeds currently active in CBiRC. For example, under the carboxylic acid testbed we have examined the production of α-olefins. We analyze the biological production of carboxylic acids (i.e., short chain fatty acids) from glucose and subsequent catalytic conversion to α-olefins through hydrogenation to alcohols and subsequent dehydration. The production cost estimate is based on the overall yields of the two processes areas and the cost of glucose feedstock. Based on theoretical carbon yields at each stage we can determine the minimum production cost of product based on feedstock cost. We then adjust the process, adding more practical yields, and modeling specific processes such as separations to add operating and capital costs. Ultimately, we can predict a minimum production cost and compare that with product cost targets to assess economic feasibility. For example, we estimate a minimum production cost for α-olefins (modeled as C8 chain length) of $0.46/lb based on an input glucose cost of $200/MT compared to an approximate market value of petroleum-derived α-olefins of $0.50/lb.

To be able to analyze a wide range of chemical pathways rapidly at early stages of development we have produced a series of generic unit process models. For each unit process (e.g., fermentation) we have developed a series of models over a range of complexity. The simplest models require little input data but yield a less certain output and more complex models require much more detailed input information, but yield more certain output predictions. We can use these models in an iterative manner in our techno-economic analysis of the testbeds, beginning with the simplest models for screening and moving to the more complex models as experimental work provides more detailed data on the likely performance of the unit processes in our testbeds.

During 2012 we developed a simplified engineering-economic model that considers how the cost of fermentation is influenced by fundamental biokinetic parameters (e.g., maximum specific growth rate, yield), to enable conversations within Thrust 2 about how metabolic engineering tradeoffs impact overall product cost. We tested the cost estimates produced by this simple model (termed BioPET - Biorenewables Process Evaluation Tool) against cost

estimates of other bioprocesses derived from sophisticated chemical process simulation tools, as well as against published literature values from the EU BREW project, and showed that BioPET produced realistic estimates given the uncertainty inherent in the results (e.g., figure below).



We presented this work to industry members who have been very interested and engaged. We also presented this work at multiple conferences.

We have also developed a set of detailed techno-economic models for a set of catalytic processes that are representative of the conversion of intermediate chemicals being developed in Thrust 2. The development of these models has answered important questions about the feasibility of scaling up such processes, and has also allowed us to develop and test our modeling and analysis techniques for the CBiRC testbed processes.

We have begun to develop a set of performance targets for the biological and catalytic conversion components of the carboxylic acid testbed. We refer to this effort as defining the "feasible space" for the testbed. We are working backward from production cost and environmental impact targets set by the incumbent processes. By modeling the performance of separations as a function of material properties we can set performance targets for conversion, such as product titer from fermentation. This sort of analysis can be performed along multiple dimensions (e.g., cost and greenhouse gas emissions) to define the performance space for fermentation that results in an environmentally and economically competitive product.

Other Relevant Work

Many organizations have made significant investments in biofuel and biorenewable chemical technologies and naturally many of these organizations are assessing the economic and technological feasibility of the technologies that they are studying. These efforts are mostly focused on specific pathways rather than taking a more general approach and assessing the potential of technology platforms and classes of biorenewable products.
There is significant interest in biorenewable chemicals and this has spawned a number of

LCAs of specific technologies. For example, a recent study examined the production of ethylene from sugar cane in Brazil. These studies are worthwhile but differ from the CBiRC efforts in LCA in that they address specific biorenewable products, rather than a platform of chemicals as is envisaged by CBiRC. We are also seeking to assess the CBiRC platform chemicals for a range of different sugar sources to provide insight as to the most environmentally attractive source of substrate.

Plans for the Next Year

Over the next year the LCA and techno-economic analysis efforts will complete the feasible space analysis of the carboxylic acid testbed (described above), and assess its value in guiding the center activities. If deemed of sufficient value, we will extend it to other testbeds.

We will also continue to revise and refine our assessments of the CBiRC testbed technologies and competing technologies, improving our analyses as the Center technologies advance. We have launched a new bifunctional molecule testbed and we will begin a series of techno-economic analyses to assess the testbed economic potential and to set development targets for the thrust teams. These analyses will provide improved information regarding technological feasibility and help identify research priorities associated with economic and environmental sustainability.

Expected Milestones and Deliverables

Two journal articles reporting the simplified engineering-economic model of fermentation and its application to the CBiRC pathways. One MS thesis on the same topic. One paper on the development of "feasible space" analysis for the Carboxylic Acid Testbed. A set of techno-economic assessments and goals for the bifunctional molecule testbed.

Member Company Benefits

Member companies gain valuable insight into the economic and environmental viability of the Center technology through the LCA and related analyses. In particular they gain economic perspective on the prospects for the testbed technologies in both near- and longer-term. Member companies also gain a detailed understanding of the economic outlook for technologies in the individual thrusts, such as catalytic processes that have been widely reported for converting biomass-derived carbohydrates to hydrocarbons. Techno-economic analyses demonstrate the major technological hurdles in these processes and identify targets for improving these processes through research and development. These targets may represent valuable research targets for member companies interested in developing these sorts of conversion processes.

Commercialization / Technology Transfer

The LCA support area provides valuable information in support of commercialization decisions by member firms and center researchers. As we develop unique data sets, such as LCI data for biorenewable chemicals we may be able to commercialize these by distributing them as an LCI database through an organization like the ecoinvent Center which distributes the ecoinvent LCA databases, which are the mostly widely used LCI databases worldwide.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **LCA 02-12F4** - Determining the Different Reaction Engineering Factors that Affect the Economic Feasibility of the Production of α-olefins from Carboxylic Acids through Techno-economic Analysis

Thrust: Research Support – Life Cycle Assessment (LCA) and Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Juan A. Lopez-Ruiz	**02/20/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Graduate Students: Juan A. Lopez-Ruiz, University of Virginia, and Sampath Gunukula, University of Wisconsin - Madison

Faculty Advisors: Robert J. Davis, University of Virginia, and Robert P. Anex, University of Wisconsin - Madison

Statement of Project Goals

The goal of this project is to develop a more detailed techno-economic analysis of the production of α-olefins from carboxylic acids and determine the influence of catalyst synthesis and morphology in activity and selectivity towards the formation of α-olefins.

Project's Role in Center's Strategic Plan

One of the integrative testbeds in the center involves the production of alkenes. Since fatty acids are readily produced by fermentation (Thrust 2), an efficient catalyst that converts fatty acids into alpha-olefins (plus CO and water) is needed.

Fundamental Barriers and Methodologies

The transformation of fatty acids into α-olefins has been studied very little compared to the conversion of fatty acids to saturated hydrocarbons. A major fraction of previous work in the area of deoxygenation of carboxylic acids uses dihydrogen, H_2, to prevent the catalyst from rapidly deactivating. Unfortunately, α-olefins are readily hydrogenated into paraffins in the presence of H_2 or isomerized in the presence of transition metal inside the catalyst pore. Furthermore, the catalyst deactivates really fast during reaction in gas phase conditions (573K and 1 bar). Thus, the work in this project is to determine the influence of catalyst synthesis and morphology in activity and selectivity towards the formation of α-olefins and address the different catalysts and reaction engineering design parameters that affect the economics of this reaction. Furthermore, we will develop a more detailed techno-economic analysis of the production of α-olefins from carboxylic acids.

Foreign Collaborations

None.

Achievements

Catalyst regeneration attempts failed. Electron microscopy and X-ray diffraction showed that metal sintering is the main cause of catalyst deactivation. Liquid-phase reaction experiments were more selective towards the formation of isomers of 1-hexene, because of the rapid metal catalyzed isomerization. Gas-phase reaction experiments were more selective towards the formation of 1-hexene. However, the catalyst deactivation was more severe under gas-phase reaction conditions.

In summary, supported metal catalysts are being modified and characterized to understand the effects of support, and preparation conditions on the TOF, product selectivity, and catalyst stability in fatty acid decarbonylation.

Plans for the Next Period

3/1/2013 – 8/31/2013:

We are working to optimize the catalyst synthesis preparation conditions to improve catalyst stability, reaction rate and product selectivity towards the formation of α-olefins. After identifying a stable catalyst, we will explore the use of solvents, and effect of concentrations on catalyst activity, diffusion, product selectivity, and catalyst stability.

After determining the effect of carboxylic acid concentration, we will identify the most important design and process parameters and develop a more detailed techno-economic analysis of the production of α-olefins from carboxylic acids. In this model, we will account for the value of co-products, reactant recycling, and catalyst regeneration.

Expected Milestones and Deliverables

The project will determine optimum conditions for catalyst synthesis to improve catalyst stability and activity.

Member Company Benefits

Members will have access to results from experimental studies of selective decarbonylation reactions catalyzed by supported metal catalysts. The member companies will also have access to catalyst synthesis and characterization techniques for supported metal catalysts.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Pre-College Learning Modules

Thrust: Pre-College Education Program

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adah Leshem	**02/28/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Adah Leshem, Iowa State University

Other Faculty: D. Raj Raman and Laura Jarboe, Iowa State University;

Other Personnel: Karri Haen, Mari Kemis, and Stacy Renfro, Iowa State University

Statement of Project Goals

CBiRC will develop, in collaboration with partnering schools and teachers, three inquiry-based learning modules for use in grades 6-12 that will introduce students to the value of biorenewables and engineering concepts.
Middle and high school teachers participating in CBiRC summer professional development programs will create Performance Tasks that will serve as learning modules relating to engineering and biorenewable topics.

Project's Role in Center's Strategic Plan

The education modules will engage pre-college students in the fields of engineering and biorenewables.

Fundamental Barriers and Methodologies

We do not believe there are fundamental barriers to this project.
The ISU Research Institute for Studies in Education (RISE) will use formative and summative assessment methodologies to evaluate the efficacy and impact of the pre-college education modules.

Achievements

- Work towards this goal began during the training workshop for the 2009 summer RET program. Teachers piloted two classroom activities: ethanol production and genetic engineering. In Summer of 2010 the modules were further developed by RET participants and GK-12 graduate students.
- During the past year (2011-2012), the modules were further developed in collaboration with the ISU Office of Biotechnology, CBiRC faculty, CBiRC lead teachers and GK12 graduate students and their middle school teach partners. Efforts were focused on the revision and modification of the ethanol activity with the addition of two more topics: biodiesel production and analysis of corn structure. Three inquiry-based curriculum units were developed and made available on the internet: **http://www.biotech.iastate.edu/publications/BiorenewablesCurriculum/**

- Over the past three years, 1500 students, ranging from middle school to graduate students, have used the biodiesel module in their classrooms or as an outreach activity. We successfully implemented the biodiesel module in 4 CBiRC partner middle schools in Des Moines, IA. Middle school student made soap as a bi-product of the experiment and used this soap to wash their hands in class.
- Equipment and materials are available via the Office of Biotechnology loan program.
- Iowa State University Bio-Economy Institute provided $5,000 to support this effort.
- During the summer of 2013 middle and high school teachers created Performances Tasks to use in their classrooms. These can be found at: https://webspace.eng.iastate.edu/cbirc/precolled/ret/summeracademy/Summer%202012%20SA%20Documents/Forms/AllItems.aspx?RootFolder=%2fcbirc%2fprecolled%2fret%2fsummeracademy%2fSummer%202012%20SA%20Documents%2fPerformance%20Tasks%202012&FolderCTID=&View={1ACE4B52-8193-48DE-B866-19AEDE911D72} and https://webspace.eng.iastate.edu/cbirc/precolled/ret/CBiRC/Shared%20Documents/Forms/AllItems.aspx?RootFolder=%2fcbirc%2fprecolled%2fret%2fCBiRC%2fShared%20Documents%2fCBiRC%20RET%202012%20Performance%20Tasks&FolderCTID=&View={29A8ED9D-6A1D-4697-B9A0-5748C179BFBC}

Other Relevant Work

- The biorenewable learning modules were presented as part of a presentation at the National Science Teachers Association (NSTA) STEM Forum and Expo in May 2012.
- Data are being collected to determine the impact these activities are having on students who conduct these curriculum modules.

Plans for the Next Five Years

The education modules will be included in more classrooms in the Des Moines school district. Workshops will be conducted during the summer professional development programs to train teachers and graduate students so that the modules can be effectively implemented in the classrooms.

Expected Milestones and Deliverables

1. Three education modules will continue to be evaluated in middle school and high school classrooms during the 2013-2014 academic year.
2. An additional education module will be developed.
3. The education modules will be demonstrated during teacher professional development workshops and GK12 training workshops.

Member Company Benefits

Providing K-12 students and teachers with educational materials associated with biorenewables will help students better understand career opportunities in the area of biorenewables and will hopefully attract students to become potential employees for member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Teacher Professional Development (RET and Summer Academy Programs)

Thrust: Pre-College Education Program

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adah Leshem	**02/28/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Adah Leshem, Iowa State University
Other Faculty: Basil Nikolau, Peter Keeling, Raj Raman and Brent Shanks, Iowa State University
Graduate Students: Michael Nolan and Lucas Showman, Iowa State University
Other Personnel: Mari Kemis, Karri Haen, Brandi Geisinger, Stacy Renfro, and Marna Yandeau-Nelson, Iowa State University

Statement of Project Goals

CBiRC will develop a long-term partnership with the Des Moines Public School District, rural school districts in Iowa and school districts in New Mexico, to provide STEM teachers (grades K-12) with knowledge, experiences, and tools to create inquiry based learning environments in their classrooms. Emphasis will be placed on teaching general engineering concepts with a strong focus on biorenewable chemicals and fuels. Teachers will be equipped to bolster a strong sense of inquiry and curiosity for science and engineering in their students. CBiRC teachers in the Des Moines Public School District will be encouraged to work collaboratively with other teachers in their district across grades and subject areas by forming science based Professional Learning Communities (PLC's).

Project's Role in Center's Strategic Plan

Providing teachers with professional development opportunities and research experiences related to CBiRC research thrusts is a central part of the Center's educational strategic plan: to prepare a strong and diverse pipeline of students committed to continuing their college education in STEM fields.

Fundamental Barriers and Methodologies

We do not believe there are fundamental research barriers to this project.
The Research Institute for Studies in Education (RISE) uses formative and summative
The ISU Research Institute for Studies in Education (RISE) uses formative and summative assessment methodologies to evaluate the efficacy and impact of the professional development programs.

Achievements

- For the past three summers (2010-2012) CBiRC has offered a one-week professional development workshop *Plants in Society* specifically designed to elementary school teachers. So far 71 elementary school teachers have participated, all of them from central Iowa including 37 from the Des Moines Public School District. The content of the workshop emphasized society's dependence on plants and the use of plants as a sustainable energy source. Funding for this workshop was provided in part by the NSF Plant Genome Research Program (PGRP). During the summer 2012 a follow-up workshop was offered in *Biorenewables* and 18 graduates of the Plants in Society workshop participated (12 of them from the Des Moines School District). The *Biorenewables* workshop, one-week in length, emphasized current research in the area of biorenewables and the value of STEM education at the K12 level. Teachers visited various related industries and met research scientists and engineers. The *Biorenewables* workshop was funded in part by Iowa's NSF EPSCoR program.
- Both the workshops are designed to provide elementary school teachers with the motivation, confidence and resources for STEM inquiry-based curriculum and instruction development. Evaluation of the workshop showed elementary school teachers scored significantly better on a content knowledge test covering plant and biorenewables topics after participating in the five-day workshops. A follow-up assessment revealed that, compared to other professional development experiences, teachers felt more inspired to integrate new content and pedagogical techniques into their curricula when basic principles were conveyed to them as community-centered ideas such as "going green." Additionally, ninety-four percent of the teachers reported changes in their perception of the nature of science four months after completion of the program.
- Over the past four summers CBiRC has offered three (2009, 2001 and 2012) three-week professional development opportunities for middle school science teachers. To date 34 middle school teachers have participated in this *Summer Academy*. Participants study the methods of biomass utilization to produce biorenewable products, including biofuels and bio-materials. This program has been supported in part by the Iowa Power Fund and the Iowa NSF EPSCoR program. Seven teachers from Des Moines public schools and two teachers from New Mexico have participated in the program.
- The CBiRC *Summer Academy* is a guided inquiry experience that provides the tools, experiences, and collaborative relationships necessary for translating the latest developments in STEM (science, technology, engineering, mathematics) into the classroom. During the summer experience, teachers learned laboratory techniques and basic biorenewables concepts through guided experimentation with CBiRC staff and graduate students. The program helps middle school teachers discover the nature of science in a research laboratory, and gives teachers a platform for building hands-on learning experiences in their science classrooms. Teachers gain real-world knowledge to share with their students and were able to relate scientific practices to issues in current events. In 2012, teachers found the *Summer Academy* program very useful. All teachers agreed that the overall quality of the program was high, that there was positive interaction with other teachers, that there was a positive relationship between the teachers and the instructor, that the program increased teacher content knowledge and understanding of renewable energy,

that participation in the program improved teacher confidence to teach students about renewable energy, that the quality of the curriculum resources was high, and that teachers will use information about renewable energy from this program in their classrooms.

- For the past two summers (2011 and 2012) CBiRC has hosted a two-day training for district science teachers at ISU campus. The focus of this event was to inform a total of 35 Des Moines Public School District science teachers (middle and high school) about cutting edge research in STEM areas. Teachers visited research labs of various on-gong projects including CBiRC labs. These training days were supported with funding from the Iowa Department of Education and in collaboration with Des Moines Public School District.
- Over the past four years (2009-2012) 38 high school science and PLTW (Project Lead the Way) instructors have participated in the CBiRC seven-week Research Experience for Teachers (RET) program including 12 teachers from the Des Moines public school district, two teachers from New Mexico (Albuquerque School District and Los Lunas School District) and one teacher from the Houston Public School District. Their research projects included:
  - Polyols to plastics: Development of catalysts and reaction systems.
  - Effect of temperature and oxygen in the reaction environment on biochar characteristics.
  - Thermo-mechanical properties of tung-oil based thermosetting polymers.
  - Bio-derived block copolymers: synthesis and characterization.
  - Nuclease mediated gene knockout in *Chlamydomonas*.
  - Atom transfer radical polymerization of acrylated epoxidized soybean oil.
  - Isoforms of 3-Methylcrotonyl-CoA Carbonoxylase of *Arabidopsis thaliana*.
- During the summer RET programs, all participating teachers attend various pedagogical seminars, workshops, and discussion groups including:
  - *Frontiers in Science and Engineering* weekly colloquium presented by CBiRC and other ISU faculty.
  - Weekly learning group discussions centered on case study development facilitated by two master teachers.
- Evaluation of the RET program produced the following outcomes:
  - Teachers expanded their knowledge of approaches to integrate collaborative inquiry-based activities in their classrooms and learned the value of keeping scientific course content current.
  - Teachers engaged in critical thinking about their teaching philosophies and methods and their impact on students' learning, including learning pedagogical methods directed by different learning styles that recognize learners as unique individuals.
  - Teachers gained collaborative relationships with other high school, middle school and university instructors to foster their continued growth in the realm of science education.
  - Teachers' teaching philosophies were particularly influenced regarding the methods used to engage students in the classroom.
  - Teachers planned to incorporate (a) hypothesis-driven lab exercises and discussions, (b) critical thinking activities, (c) long-term experiments to engage students in continuous active learning, and (d) the development of analytical skills in their classroom curricula.
  - Participants gained laboratory skills, a better understanding of scientific inquiry, persistence and patience in the laboratory setting, and confidence working in a research environment.
  - Participants gained a better understanding of biorenewables and CBiRC's research goals.

- Selected teacher quotes relating to the summer programs:
  - *It gives our students a chance to see us being enthusiastic about science, which is contagious…that enthusiasm is contagious.*
  - *There are so many resources…there are just so many things we didn't know about in our own community. They're really at the forefront in the bio-renewable effort…all these kids had the impression that if you want to get a good job in a science field it's something that's far away. And it's like, "No it's not, it's right here," you know, "It's right in your backyard." Here we have engineers doing their own—creating their own vehicles that have never been created before to harvest and those kinds of things right there…So the potential for opportunities for our students immediately staying in their own communities and maybe even moving back out repopulating the rural areas is really moving for me.*
  - *This is going to give us an opportunity to talk to our kids about job careers and get them enthusiastic.*
  - *I think the thinking skills [were] really something that was reinforced for me. Having taught reading, I know that those are important in that area and I knew—I thought—it was in science and I tried to include those a lot, but this was reinforcement that they need to keep pushing.*
  - *It's a great opportunity to do things like this with a group of your peers. [Having a] dialogue with your peers makes you rethink how you want to do things and what you want to accomplish. I think it's very energizing.*
- Presentations were delivered at the National Science Teachers Association (NSTA) STEM Forum and Expo conference, May 2012. The topics presented were CBiRC's *Summer Academy* and *Plants in Society*.

Other Relevant Work

- The evaluation team is examining results that show that standard assessment of skills gains using self-efficacy measures did not show significant longitudinal gains in a professional development program for high school science teachers (meaning that first year participants reported significant pre-post gains, but did not necessarily in subsequent years of participation). The team is using an authentic experimental design task that addresses difficulties in estimating increased science research skills for returning science teachers. Preliminary data suggest that this method may be informative for measuring science skills gained in diverse research-based professional development programs, that the relationship to science process self-efficacy is unclear, and that teacher acknowledgement of creativity as part of the experimental design process may impact results.
- RET participants are implementing the case studies they developed during their summer professional development. Teachers have stated the case study technique was an easy way to translate the CBiRC research experience to the classroom.
- RET teachers participated in a philosophy of science study that included the Views on Science and Education (VOSE) questionnaire. The results will be used for continuing research and publication in the area of teacher perceptions of the nature of science.
- RET participants are encouraged to bring their students to visit CBiRC at the ISU campus. To date over 100 middle and high school students have visited CBiRC facilities on the ISU campus.

Plans for the Next Five Years

- Measure student impact as a result of teacher participation in the RET program.

- Conduct follow-up surveys with the teachers who participate in the professional development programs to determine the program effects on their teaching and their students learning during the ensuing academic year.
- CBiRC will extend the RET program to include more participants from rural Iowa and New Mexico as well as teachers from the Des Moines Public School District.

Expected Milestones and Deliverables

1. An article is in preparation about evaluation of the RET program using design assessment.
2. Presentations of CBiRC's teacher professional development programs will be submitted to the AAAS International Teacher-Scientist Partnership Conference, February 2014 and ASEE June 2014.
3. CBiRC will continue to offer K12 teacher professional development opportunities.

Member Company Benefits

The middle and high school teachers who participate in CBiRC professional development programs will be able to help their students better understand career opportunities in the areas of engineering and biorenewables and will hopefully attract students to become future scientists, engineers, and potential employees for member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Young Engineers Program

Thrust: Pre-College Education Program

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adah Leshem	**02/11/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Adah Leshem, Iowa State University

Other Faculty: Basil Nikolau, Brent Shanks

Graduate student: Mike Nolan, Jennifer Chmielowski

Other Personnel: Mari Kemis, Karri Haen, Brandi Geisinger, Stacy Renfro

Statement of Project Goals

Provide 10^{th}-12^{th} grade high school students the opportunity to participate in CBiRC related research projects as well as non-associated engineering and scientific research projects.
Provide pre-college students exposure to both academic and career options in science, technology and engineering fields.

Project's Role in Center's Strategic Plan

Participation in CBiRC related research projects will engage pre-college students in the fields of engineering and biorenewables.

Fundamental Barriers and Methodologies

We do not believe there are fundamental barriers to this project.
The ISU Research Institute for Studies in Education (RISE) will use formative and summative assessment methodologies to evaluate the efficacy and impact of the pre-college education modules.

Achievements

The CBiRC Young Engineers program has expanded and currently offers research internship opportunities to 10^{th}-12^{th} grade high school students in the physical and life sciences as well as non-CBiRC associated fields of engineering. The program is called *Young Engineers and Scientists* (YES) and is offered year round. Students who participate in the fall and spring semesters are typically from local high schools and do not receive stipends but instead receive high school credit listed as "independent research study" on their high school transcript. Students who participate in the summer YES program are recruited from ISU's Science Bound program that works closely with Des Moines and other central Iowa school districts' underrepresented minority students to encourage and support their preparation for and pursuit of an academic degree in STEM. These students receive a stipend but do not receive credit. All students prepare and present a poster outlining their research project. All students who participate in the summer program complete

periodic assessment surveys. Over the past three years a total of 16 high school students participated in the summer YES program.

- CBiRC continues its collaboration with ISU's Science Bound program to recruit URM rising seniors from central Iowa high-need school districts to its summer programs. In summer 2012, the YES program hosted eight rising high school seniors, six of whom were URM students.
- Three of the 2011 summer YES participants (all URM) are currently enrolled at ISU, majoring in chemical engineering, aerospace engineering and mechanical engineering. The fourth student is currently a senior in high school.
- Three of the four 2010 summer YES participants (all URM) are currently in their sophomore year at ISU majoring in a STEM field.
- Program evaluation findings show that students participating in the summer programs have:
  - A deeper appreciation for science and scientists.
  - Self-confidence in their ability to conduct research.
  - Knowledge of different fields of science.
  - A better understanding of academic options.
  - A stronger interest in pursuing a research career.

- 2012 students' comments provide insight into their experience.
  - *I worked with a graduate student, and he was always there to help us (us meaning me and the other teacher that [he] was working with). Sometimes he would give us direction and let us go from there. That really helped me figure things out on my own rather than going back to him and I liked that I liked doing things on my own.*
  - *I know at high school for our experiments the teachers tell us the results, like what we are trying to look for and that is no fun whatsoever. They try to hold our hands through it and say this is what you are going to do, and this is what is going to happen. If something else happens, you get it wrong; you do it over again until you get it right. During YES, it is very different. They told us what the results they were hoping for but if we got any others to record that. We do not really know if different data is a mistake or not a mistake. It is really good to find results and start comparing them and actually putting things together by only the data that is being collected and to pull out our own answers.*
  - *Research requires patience. You always wait for the reaction to finish and if it messes up you have to go back and do it over again and wait more. Oh, and research requires background information. You are given a topic and you have to go back and read and research what other people have done in the same area. If no one else has done it, you have to think of ways to do it. You know a new way, your own way so it is a different level of technicality.*
  - *It feels good to be around someone who is at your own level of thinking or higher. It just makes you more comfortable. In high school, we're all stuck together and some people don't understand things as quickly as you do.*
  - *I really like the diversity in the labs because there are a bunch of people from different nationalities and different countries and it's just bringing the world together and I think that's a really good thing to do especially in today's society.*

- Interviews with four YES students currently in their first year at Iowa State University revealed that YES was valuable in helping them make choices and be successful early on in their college career.
  - *Participation as part of a research team was seen as a legitimate experience, in which they were treated as a peer in the laboratory and able to make a contribution.*
  - *They understood the value in the opportunity to interact with experts and knowledgeable*

others.
  - *The poster presentation required them to produce a high quality, professional product, using multiple methods of communication.*
  - *The YES experience solidified their decisions to pursue STEM field and affirmed or refined their choice of major.*
  - *They were prepared for college, in that they were comfortable on campus, ready for college-level study, and had increased academic and STEM self-efficacy.*

- At one-year following participation in the summer YES program, approximately 90 percent or more students reported that they had opportunities to conduct research about a topic they learned in class, gain research skills, gain experience that would help them get into college, learn about a career in science/engineering, and receive help in deciding on a college major and a career in science/engineering.

Plans for the Next Five Years

- CBiRC will continue the Young Engineers & Scientists program to offer high school students research internship opportunities.
- CBiRC will continue to collaborate with Science Bound, Iowa State University's premier pre-college program for underrepresented minorities, to increase the number of ethnically diverse Iowa students who pursue ASTEM degrees.
- CBiRC faculty at partner institutions will be encouraged to mentor high school students as part of the CBiRC YES program.

Expected Milestones and Deliverables

1. The high school students who participated in the Young Engineers & Scientists program at Iowa State University will present posters outlining their research projects at a poster receptions either at Ames High School in May 2013 or at the conclusion of the 2013 summer RET program.
2. A number of the students will present at local regional state Science Fairs.

Member Company Benefits

Providing pre-college students exposure to how research is conducted in the field of engineering will help students better understand career opportunities in this area and will hopefully attract students to become potential employees for member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Symbi, Iowa's GK-12 Program: Growing Iowa's Scientists for a Greener Tomorrow

Thrust: Pre-College Education Program

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adah Leshem	**02/28/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Basil Nikolau, Iowa State University

Other Faculty: Adah Leshem, D. Raj Raman, Iowa State University

Graduate Students: Christian Bischoff , Thomas Garrison, Peter Hondred, Brandon Jeffery, Robert Literman, Tim Mitchell, Mike Nolan, Lucas Showman, Paul Skrade, Ryan Swanson Iowa State University.

Other Personnel: Karri Haen, Heidi Hain, Mari Kemis, Diana Loutsch, Stacy Renfro, Jordin Schweitzer, Stephanie Zywicki, Iowa State University; Kim O'Donnell, Des Moines Public School District

Participants: Amy Kissell and Anna Lund, Brody Middle School; Justin Blietz and Luke Spencer, Harding Middle School; Maureen Griffin, Eric Hall and Katherine Larson, Hoover High School; Jacqui Stewart, Hoyt Middle School; Debra Victor, McCombs Middle School; Gary Morris and Tim Weida, Meredith Middle School

Statement of Project Goals

Engage graduate students conducting interdisciplinary research in the area of biorenewables, with Des Moines, IA, middle and high school educators, students and their parents, and administrators. The objectives of this engagement are to: 1) provide graduate students with the skill sets and communication proficiency to explain their science and illustrate core STEM principles to a young and receptive audience; and 2) provide middle school students exposure to inquiry-based learning experiences and authentic demonstrations of mastery of core concepts.

Project's Role in Center's Strategic Plan

Provide graduate students and K-12 teachers with professional development opportunities, specifically to become better communicators of STEM subjects.
Provide pre-college students with exposure to CBiRC and the value of biorenewables and engineering concepts.
Provide STEM content professional development to participating middle and high school science teachers.

Fundamental Barriers and Methodologies

We do not believe there exists to be a fundamental barrier to this project.
The ISU Research Institute for Studies in Education (RISE) uses formative and summative assessment methodologies to evaluate the efficacy and impact of the program on GK-12 Fellows, middle school teachers, and middle school students.

Achievements

- CBiRC was awarded Iowa's first GK12 grant in May 2010.
- Iowa's first GK12 program was branded with the name *Symbi* and a website was developed: **www.gk12.iastate.edu/**
- To date 18 STEM graduate students have filled the position of GK12 Fellow (seven of these graduate students have repeated the program).
- To date 15 science teachers from five middle schools and one high school in the Des Moines school district, have participated in the Symbi program as GK-12 teachers, impacting approximately over 2,000 students each year. Four of these schools are minority-serving institutions.
- In May and April 2012 Symbi hosted Science Days at two middle schools in Des Moines. These events took place in the schools' gym and featured on-going presentations and demonstrations by ISU STEM graduates students and local industry. All the students in these schools spent their science class period visiting with the presenters to learn more about different subject areas in STEM and possible STEM career paths. Over 1,000 students participated in Symbi Science Days.
- The Fellows and Teachers completed training in Summer 2012 in preparation for classroom collaboration and attended the graduate course *Symbi Professional Practices Tutorial,* CI 593A, taught by Dr. Denise Crawford in the ISU Dept. of Curriculum and Instruction.
- To assess and improve the communication skills of the Fellows, ISU Department of English professor Amy Slagell presented a workshop on communication effectiveness in the classroom. A doctoral level graduate student in School of Education, ISU, was hired to observe the Fellows in the classroom and provide individualized and group feedback about how to improve classroom communication effectiveness, specifically with regard to science and engineering topics covered in the classroom. Classroom observations were conducted at the beginning and end of the school year. Fellows were observed using a rubric that assessed their organization, use of scientific vocabulary, equity in interaction with students, delivery, use of technology, use of questions, and ability to create student engagement. Initial analysis indicates the Fellows' level of performance was above average in all categories. Additional analysis comparing classroom communication will occur after the final observations in April 2013.
- Fellows are required to give a ten-minute research presentation to non-science major undergraduates. Fellows give these presentations before they begin their work in K12 classrooms and then again at the end of the year. The pre- and post-presentations are video recorded. A doctoral student in Technical Communications, Department of English, ISU, was hired to observe the video recordings and compare pre- and post-presentations. Results of this study are currently being analyzed.
- Monthly surveys of the Fellows and Teachers indicate that *Symbi* is meeting all project

objectives. Middle and high school students are very receptive to the presence of the graduate student "resident scientists/engineer" in their classrooms. According to one teacher, "Students enjoy interacting with the fellow. He lends a unique perspective and does a nice job of communicating his ideas in an interesting way to students. He does a nice job making things understandable for my students. According to my student, "He's somebody who has been in the field. He gets us to learn a little more." Added another student, "He makes it more interesting to learn." (GK12 teacher comments)

Symbi Teachers report that their students are more engaged and are asking more questions than in previous years. *Symbi* Teachers also report that the graduate students have demonstrated improved communication skills over the past semester. "I think the Fellow has done an excellent job of taking his research and other difficult topics and relating them to the middle school students. He tries to present things in a way that is relevant to the students and engaging. I think being in the program for a second time as well as writing his blog that is tailored to middle school students has really helped him. His interactions with the students are great." (GK12 teacher comments).

- Practiced lesson plans and projects have been developed as a result of the Fellow and Teacher collaboration in the classroom. These lesson plans and projects are posted on the *Symbi* website and available to the public to print and implement into their classroom: http://www.gk12.iastate.edu/classroom_projects/lesson_plans.asp

Other Relevant Work

- Data are being collected from middle/high school students, Fellows, and Teachers to determine the impact *Symbi* is having on middle school students. At this time, data have been collected from approximately 1000 students (Fall 2010, Spring 2011, Fall 2011, Spring 2012, Fall 2012), with 611 matched responses. Data were collected from over 800 students in Fall 2012, including at least 70 students who have matched data from the previous year (meaning that they have been in classes with more than one GK12 Fellow and Teacher).
- Student attitude data toward science and science as a career have also been collected during early Fall semester 2012 from Hoover High School (Des Moines, IA) science students and will be collected as a post-survey in April 2013. It is hypothesized that student attitudes and career aspirations can be significantly impacted in classrooms with resident scientists and/or teachers who have had specific professional development in inquiry-based learning and laboratory experience. Changes in attitude and career aspiration will be analyzed by three groups—students with a GK12 Fellow as resident scientist in the classroom, students with an RET teacher, and a control classroom (no resident scientist and no or limited professional development). Analysis and continuing data collection will take place during Year 3 of the GK12. Ongoing tracking of students post-graduation will occur to see any future influence of the program.
- Data are being collected from the major professors supervising the Fellows to measure influence of participation in the GK12 Program on Fellows' ability to communicate research, ability to communicate science concepts, research productivity, mentoring other students in the lab, outreach to K-12 students, and developing transferrable career skills.

Plans for the Next Five Years

- As this program nears the end of its NSF funding (2015) discussions with university

administration and the Des Moines school district are underway to negotiate plans to institutionalize the Symbi program.

- During the next two academic years Symbi will continue to offer nine graduate fellowships and will continue to collaborate with the Des Moines public school district.
- Continue to improve GK-12 training program based on program evaluations and assessments.
- Plan and implement a *Symbi Science Day* at three partner middle schools in Des Moines to create events for all students at each school to learn about ASTEM (Agriculture, Science, Technology, and Mathematics) research and careers.
- Continued data collection to examine middle school student achievement, attitudes toward science, and career plans in STEM longitudinally.

Expected Milestones and Deliverables

1. Continued growth of the *Symbi* program with more teachers, middle/high school students, and graduate students involved.
2. Greater involvement with the Des Moines community through events like Science Days.
3. Further development of the Symbi lesson plans and projects will be used in other CBiRC programs and made available to the all CBiRC teachers as a way to directly involve their students in hands-on learning and discovery activities.

Member Company Benefits

Symbi will invite member companies to exhibit their products at Symbi Science Days. Symbi Fellows will accompany the middle school students during the school science days to help them better understand career opportunities in the area of biorenewables and will hopefully attract students to become potential employees for member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: CBiRC Graduate Minor & Graduate Certificate (for extension to partners)

Thrust: University Educational Programs

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Dave Raj Raman	**03/04/13**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Raj Raman, Iowa State University

Other Faculty: Brent Shanks, Iowa State University

Other Personnel: MaryAnn Moore, Brandi Geisinger, and Peter Keeling, Iowa State University

Statement of Project Goals

The minor in Biorenewable Chemicals allows students from a variety of allied disciplines to understand the opportunities for developing biorenewable chemicals via a combination of biocatalytic and chemical catalysis steps. In addition, students in the minor get explicit entrepreneurial internship training, a background in the general issues related to production and processing of biorenewable resources, and exposure to the economic and environmental realities of the chemical industry. The interdepartmental minor resides within the Graduate College and is formally affiliated with CBiRC. The minor consists of a 14-credit hour sequence: 8 hours of graduate coursework encompassing *Fundamentals of Biorenewable Resources and Technology* (3 cr), *Biological and Chemical Catalysis* (3 cr), *The Evolving Chemical Industry* (1 cr), and *Entrepreneurship in Biorenewable Chemicals* (1 cr), plus 6 credits of coursework selected from a list of courses reflecting CBiRC's three technical thrust areas:

- Thrust 1: New Biocatalysts for Pathway Engineering
- Thrust 2: Microbial Metabolic Engineering
- Thrust 3: Chemical Catalyst Design

Additional training of students in the graduate minor occurs through the annual CBiRC center-wide meeting where students will present posters and learn about each other's research findings, and thereby gain a better appreciation for both chemical and biological catalysis routes for producing biorenewable chemicals.

Because CBiRC partner institutions lack the faculty numbers (and institutional structures in some cases) needed to institute stand-alone graduate minor programs, a Graduate Certificate in Biorenewable Chemicals program was also instituted.

The disciplines of biological and chemical catalysis have traditionally been separate. And while some of this separation will always exist, the core mission of the NSF Engineering Research Center for Biorenewable Chemicals (CBiRC) is to transform the chemical industry by integrating biological and chemical catalysis systems to create a generalized framework for producing biorenewable chemicals. Graduate education is central to achievement of this

mission, because graduate students will develop the expertise needed to drive future research programs in this area, both in academic and industrial settings. The minor and certificate furthers CBiRC's mission by producing disciplinary experts from programs like Chemical Engineering, Chemistry, and Biochemistry, Biophysics, and Molecular Biology, who are interdisciplinary trained to become globally-competitive college graduates capable of designing integrated chemical/biological processing systems.

Project's Role in Center's Strategic Plan

The minor and certificate programs are central to CBiRC's strategic plan to educate graduate students in this area.

Fundamental Barriers and Methodologies

Declaring a minor at Iowa State University required the approval by all departments or sponsoring groups (five curriculum committees, five department heads), the appropriate college curriculum committees (two), the college faculty for one of the colleges, the college deans (two), the Faculty Senate Curriculum Sub-Committee, the Dean of the Graduate College, and the Executive Vice President and Provost. We have surmounted this barrier, but have a second, deeper barrier, which is that talented graduate students (and their CBiRC-affiliated major professors) may feel that the return on investment (of student time, associated with additional coursework need for the minor) is not sufficiently large, and the program may fail to have a significant number of students.

Although mechanisms exist to offer all of the four core courses to students at all partner institutions, graduate minor degrees cannot be conferred by ISU to non ISU students at the partner institutions. So a major barrier to making this graduate education available to **all CBiRC graduate students**, and not just those at ISU, is the non-transferability of the graduate minor. To overcome this barrier, we are making a Graduate Certificate available to all non-ISU CBiRC graduate students. The coursework requirements are identical (8 credits of core coursework, taken via distance methods) plus six semester hour equivalents of thrust-specific courses, taken at the home (i.e., partner) institution. The certificate, which does not carry a university seal, is granted by CBiRC, and consists of a formal "certificate" and a letter from the CBiRC University Education Program Director, detailing the coursework taken to achieve the certificate, and the learning objectives of the program. The latter document can then be used by students as part of their applications for jobs.

Achievements

Since the initiation of the graduate minor program, all of the CBiRC core courses have been taught at least once. *Catalysis and catalytic processes*, BR C 688, focused on the fundamentals of hetero-geneous and bio-catalyst synthesis, characterization and reaction testing, was first offered in the spring of 2010. Nineteen students from three universities (ISU, the University of Virginia, and the University of New Mexico), participated in the course. Of these, nine students were affiliated with CBiRC. Students commented that they found both the broad overview and particulars of the course (derivation of equations, specific research examples from the literature, industrial applications, etc.) very pertinent to their research and potential careers. The course is being taught at the time of this writing (Spring 2013), this time to 17 total students from a range of majors, including Chemical Engineering, Chemistry, Mechanical Engineering and Biochemistry.

BR C 506, *The Evolving Chemical Industry*, was offered during the summer of 2010 with distance education opportunities for students at partner universities. The course was designed in order to

help students gain an understanding of the current chemical industry and its development, with special emphasis on the commercialization process of biorenewable chemicals. Seventeen students from CBiRC partner institutions were enrolled in the course: 15 from ISU and two from other universities. Evaluations showed students felt very strongly that this course helped them gain an understanding of the importance of economic and environmental constraints in the practice of engineering. This course will again be offered in August 2013.

The graduate minor program offered a new 1 credit course in spring 2011 (and again in spring 2012 and 2013), BR C 507, *Entrepreneurship in Biorenewable Chemicals*. This course was designed to develop an understanding of discovery research and its relationship to entrepreneurship and innovation in the broad area of biorenewables. Participants of the course learn the critical importance of developing a sound techno-commercial analysis and evaluation of intellectual property, as well as learn how to utilize local resources in entrepreneurship. The course objectives include teaching students how to define key assets, write a business plan, and how to take the necessary steps to go about founding a company and securing research funds.

Other Relevant Work

We are unaware of any other graduate programs in biorenewable chemicals.

Methods developed as part of a USDA Higher Education Challenge Grant to develop a Virtual Education Center in Biorenewable Resources (PI: Raman) heavily used in CBiRC's graduate minor efforts. Specifically, the USDA project Virtual Education Center model relies upon sharing video lectures – rather than the onerous moving of student credit hours across institutions – to allow instructors at multiple sites to contribute their expertise to a course. In the case of the Graduate Minor in Biorenewable Chemicals, all four of the core courses are using this model, with additional lectures from Distinguished Regents Professors Abhaya Datye (University of New Mexico) and Earnest Jackson Oglesby Professor Bob Davis (University of Virginia).

Plans for the Next Year

Oversee and grow both the minor and certificate in Biorenewable Chemicals.

Expected Milestones and Deliverables

Over the next five years, at least 15 PhDs will graduate with a minor or certificate in Biorenewable Chemicals. The graduate minor program currently has five ISU graduate students enrolled. The graduate certificate program currently has five non-ISU CBiRC graduate students enrolled.

Member Company Benefits

The graduate minor is the culmination of CBiRC's educational mission, and the part of the educational programs most likely to *directly* impact member companies by training outstanding engineers (and, in CBiRC's case, scientists) who be employed as interns or permanent employees at member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: CBiRC Research Experience for Undergraduates (REU) Program

Thrust: University Educational Programs

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Dave Raj Raman	**03/01/13**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Raj Raman, Iowa State University

Other Faculty: Brent Shanks, Basil Nikolau, Laura Jarboe, George Kraus, Jackie Shanks, Tom Bobik and Keith Woo – Iowa State University; Ka-Yiu San and Ramon Gonzalez – Rice University; Bob Davis and Matt Neurock – University of Virginia

Graduate Students: Shivani Garg, Ting Wei Tee, Ping Liu, Liam Royce, Jason Anderson, Gerry Pollock, Alexis Campbell, Gina Roberts, and Mark Brown – Iowa State University; Hui Wu and Maria Rodriguez – Rice University; Matthew Ide – University of Virginia.

Other Personnel: MaryAnn Moore, Karri Haen, Mari Kemis, and Marna Yandeau-Nelson, Iowa State University

Statement of Project Goals

The CBiRC REU program strives to recruit, nurture, and train the next generation of creative and adaptive engineers who will be capable of bridging the gap between chemical and biological catalysis. In so doing, we hope to produce technical professionals capable of moving the US chemical industry toward a more sustainable model of production based on biorenewable feedstocks though experiential learning in CBiRC faculty laboratories. During the course of the CBiRC REU program, opportunities exist for student specialization of research in one or a combination of CBiRC thrust areas: biocatalysts for pathway engineering, microbial metabolic engineering, chemical catalyst design, or life cycle analysis of biorenewable chemicals. The program further integrates hands-on research with a series of weekly lectures or other center-wide interactions, which include lab tours, workshops, and meetings, and the opportunity to present student project results to the CBiRC community. CBiRC educational programs strive to instill students with a multidisciplinary background, so that they can devise creative approaches to solving engineering problems, including recognizing the wide-ranging potential for both chemical and biological catalysis for the production of environmentally sustainable chemicals. Further, students are engendered with the understanding that economic, environmental, and ethical constraints are central to the practice of engineering, and, thus, CBiRC engineers should be capable of evaluating their work based upon these criteria.

Project's Role in Center's Strategic Plan

The Research Experiences for Undergraduates program (REU) is a major component of the CBiRC university education program, which particularly focuses on undergraduate student training.

Through participation in the REU program, science and engineering students develop their skills through experiential learning with the Center's interdisciplinary research. Guided by CBiRC faculty, postdoctoral research associates, and graduate students, undergraduates become a part of a team involved in the development of new integrated catalytic systems for the conversion of bio-based feedstocks to industrial chemicals.

Fundamental Barriers and Methodologies

Due to the nature of this project, there are not fundamental research barriers per se. However, there are challenges associated with the establishment of large programs that serve multiple universities. Since the summer of 2010, the CBiRC REU has grown to be a multi-institutional program, thus creating logistical challenges associated with the coordination of students and programs among the participating universities. Some of these challenges are straightforward, such as obtaining IRB approvals for continued evaluation of CBiRC-affiliated undergraduate students. Other issues are implicit to any program that has partners which do not exist locally: extending the program to partners has encouraged us to determine how to better coordinate laboratory safety training, general program orientations, and other center-specific meetings, such that students do not have to participate in these exercises more than once. All of these issues were addressed in 2010 and again in 2011. In 2010, although we had an academically strong cohort, friction between two REU participants and each other, as well as with their host labs, caused a general frustration in the group that was difficult to overcome. In 2011, we instituted pre-offer interviews of all students, in which personal interaction styles were probed. Furthermore, during interviews and again during orientation, the importance of collegiality in host labs and between REUs was emphasized. Due to these actions (and perhaps also through fortuitous selection), the 2011 REU was extremely successful. We promulgated a mentoring podcast in 2012, and followed viewing of that 13 minute piece with a face-to-face or electronic meeting with potential mentors; the results were excellent: we had a highly successful 2012 REU. We are on track to do similar in 2013.

Plans for the Next Year

Over the next four years the CBiRC REU program will graduate an additional 50 students (for a total of 80+ by March 2016), with 15 – 20% of these students having spent the majority of their summers at partner institutions. The progress of these 80+ graduates will be monitored to the best of our ability, and we expect that at least 40 of our graduates will go on to graduate school, with half of those going to fields relevant to biorenewable chemicals. Multiple CBiRC REU alums have already gone on to graduate programs in chemistry, with the intent to work on biorenewable chemicals. We will continue our aggressive efforts to recruit from under-represented populations and have recently made strong contact with faculty at Fort Valley State University and are hopeful that this will lead to a long-term relationship that will both bring REU students *and* enable FVSU faculty development in the area of biorenewable chemicals. We are leveraging our educational hypotheses regarding mentoring into a study of mentor actions that lead to student success, and anticipate producing a refereed publication in this area in 2013.

Expected Milestones and Deliverables

Out of the 14 students anticipated for the 2013 REU program, we expect to have 10 of those students remain at Iowa State University throughout different research projects and 3-5 students attending partner institutions after they complete orientation at Iowa State. They will work on interdisciplinary teams with faculty, graduate students, post-docs, and in some cases industrial

partners. They will also engage with students participating in other Iowa State University based REU program in seminars, short courses, research tours, field trips and social events with mentors, graduate students, postdoctoral associates and others involved in the research of biorenewables. Students participating in the CBiRC REU program will be expected to work in a research lab for 40 hours per week for 10 weeks, participate in weekly lab meetings and all other scheduled events. At the end of the program they will be required to present their research findings both orally and in the form of a poster.

Member Company Benefits

Potential exposure to creative and adaptive engineers capable of bridging the gap between chemical and biological catalysis.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Iowa State Coleman Faculty Entrepreneurship Fellow (*a sponsored project*)

Thrust: University Education Program and Innovation Ecosystem

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Peter L Keeling	**02/28/2013**	**03/01/2011 to 02/28/2013**

ERC Team Members

Project Leader: Peter Keeling, Iowa State University

Other Faculty: D. Raj Raman, Iowa State University

Statement of Project Goals

The project goal was to create and develop a course in Technology-Led Entrepreneurship related to Biorenewable Chemicals. This was initially done within the framework of the Coleman Fellows program in order to learn from other experiences in teaching and fostering entrepreneurship in academia. A fundamental mission of the course is to show that entrepreneurship is not the sole realm of business school graduates. Instead technology-led entrepreneurship is shown to be a fundamental skill that all scientists and engineers should strive to acquire. The course curriculum is now formally recrafted around the "Business Model Canvas" used in the I-Corps Program.

Project's Role in Center's Strategic Plan

The Entrepreneurship Course was designed as an addition to the CBiRC Graduate Minor in Biorenewable Chemicals. The course contributes to the Center's strategic plan by developing an improved understanding of entrepreneurship at the graduate student level. The Graduate Minor allows students from a variety of allied disciplines to understand the opportunities for developing biorenewable chemicals via a combination of biocatalytic and chemical catalysis steps. In addition, students in the minor gain explicit entrepreneurship experience, a background in the general issues related to production and processing of biorenewable resources and exposure to the economic and environmental realities of the chemical industry.

Fundamental Barriers and Methodologies

The Entrepreneurship Course program overcomes traditional barriers and misconceptions that entrepreneurship is difficult and only for wealthy extravert superstar entrepreneurs. The course impacts students by teaching them that entrepreneurship is just as much a part of existing businesses and academia as it is about forming a startup entity. The course impacts the Center by forming a foundation of understanding of the principles of entrepreneurial practices and how this must be firmly embedded in technical and commercial analyses of any new innovation or biorenewables opportunity. The course introduces graduate students to discovery research and how this is evaluated as a risk-reward equation. It is expected that this will have a trickle-effect into the CBiRC faculty. The course shows how new knowledge leads to innovations and inventions that must secure sustained funding to become valuable. It introduces students to the process of going about

seeking initial funding from various sources including the SBIR program as well as State opportunities, Angel funds and Venture Capital funds. This process is demonstrated to be fundamentally similar to the process of project evaluation in large companies. The course proceeds with a step-by-step review of how to put together professional business/project plans and presentations in order to position a startup entity or new project for sustained ongoing funding.

Achievements

In 2011, the course had 14 graduate students, in 2012 there were 13 students and in 2013 there were 11 students (total 37 graduate students). All students completed the course and some went-on to found a company. One entity won an I-Corps grant which started in March, 2012 and subsequently applied for an STTR. Three startups were awarded funding from the State of Iowa. All CBiRC startups are encouraged to take the mentoring program in CBiRC's Biobased Foundry.

Other Relevant Work

There are many types and levels of entrepreneurship that are traditionally designed within the university business school curriculum. The CBiRC course focuses on Technology-Led Entrepreneurship and is a fundamental and vital departure from this traditional business-led realm.

Plans for the Next Year

The course is planned to continue indefinitely within the Graduate Minor in Biorenewable Chemicals. In 2012, the course became a formal course requirement for another graduate minor entitled "Biorenewable Resources and Technology".

Expected Milestones and Deliverables

The course stimulates and nurtures an entrepreneurial spirit within CBiRC. We hope we can stimulate at least 2 new startup companies each year.

Member Company Benefits

Industry members are inherently risk-averse. Yet they can gain from this, because startup entities are better able to deal with risk. They can become sources of funding and become strategic partners or mentors as well as customers. The industry members could eventually become owners of these startup entities.

Commercialization / Technology Transfer

Translational Research Grants are a superb source of funding for early stage startup entities. Creating startup entities is a fundamental metric of the Gen-3 ERC's.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

The following is actually an associated project. However, for Gen-3 ERCs, foreign partner associated projects may include a project summary rather than only an abstract if the project is of particular importance to achieving the vision of the center.

Project Title: **PIRE**: Molecular Engineering for Conversion of Biomass-derived Reactants to Fuels, Chemicals and Materials

Thrust: International Education Program

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Abhaya K. Datye	**02/28/2013**	**03/01/2011 to 02/28/2013**

ERC Team Members

Project Leader: Abhaya K. Datye (U-NM)

Other Faculty Investigators: Brent Shanks and George Kraus (ISU); James A. Dumesic (U-WI); Matthew Neurock and Robert Davis (U-VA); Robert Schloegl, Mathias Scheffler and Malte Behrens (Fritz Haber Institute of the Max Planck Society); Markus Antonietti (Max Planck Institute for Colloids and Interfaces), Ib Chorkendorff and Thomas Hansen (Technical University of Denmark) Stig Helveg and Esben Taarning (Haldor Topsoe A/S), Hans Neimantsverdreit and Peter Thune (Technical University Eindhoven, Netherlands), Bert Weckhuysen, Krijn de Jong and Harry Bitter (Utrecht University, Netherlands) Leon Lefferts (University of Twente) and Dmitry Murzin (Abo Akademi, Finland).

Post-doctoral Associates: Barr Halevi and Hien Pham (UNM)

Graduate Students: Andrew De La Riva, Angelica Benavidez, Jonathan Paiz, Eric Petersen and Tyne Johns (UNM); Juan Lopez-Ruiz, Matthew Ide, Joe Kozlowski, David Hibbitts, Craig Plaisance, Derek Falcone (UVA); Ryan Snell, Michael Nolan, Thomas Garrison, Jason Anderson, Keenan Deutsch and Jennifer Lee (ISU); Elif Gurbuz, Drew Baden and Carrie Ferberow, Ronald Carrasquillo (U-WI)

Undergraduate Students: Amanda Anderson (UNM), Elliott Combs (ISU)

Statement of Project Goals

This Partnership for International Research and Education (PIRE) brings together together four U.S. and eight European institutions to investigate critical steps required for chemical transformation of biomass-derived reactants into useful products. The five year plan for collaborative research focuses on metal-catalyzed conversion of carbohydrates and their derivatives to chemicals, fuels and materials. The educational aspects of the collaboration draw upon the shared intellectual and physical resources of each partner to provide multi-faceted international experiences for U.S. graduate and undergraduate students and post-docs. The resulting internationally distributed, virtual

center helps prepare a new generation of globally-engaged science and engineers while the research partners pursue compelling research questions associated with biomass conversion and enhanced engineering of metal catalyzed reactions.

The PIRE network brings together complementary strengths, for instance the U.S. partners specialize in aqueous phase processing, microkinetic modeling, and kinetic and mechanistic characterization of catalysts. The German counterparts are well known for novel catalyst synthesis and modeling of chemical reactions. Danish partners bring strengths in surface science approaches to studying new catalysts and theoretical expertise in modeling catalytic reactions. The groups in Netherlands are well known for development of in-situ spectroscopic techniques and Finland is known for its research on wood chemistry, due the plentiful supplies of woody biomass. Together, the University of New Mexico-led PIRE team works to achieve conversion of specific C-C or C-O bonds in the presence of multiple similar functional groups and to improve our understanding of:

1) adsorption of molecules with a high level of functionality on metal surfaces;
2) the role of water or solvent in liquid phase processing; and
3) how to build in hydrothermal stability into catalysts.

The results will lead to innovative molecular engineering for conversion of biomass-derived reactants to fuels, chemicals and materials.

Sustainable production of chemicals, materials and energy from renewable resources provides a rich source of research problems that can be integrated with the education of students participating in PIRE activities. This model includes international mentoring, research internships and summer research for U.S. graduate and undergraduate students, as well as summer schools and course development. Overall, results stemming from this PIRE fulfill the program objectives of building international partnerships that advance research and provide innovative educational opportunities through valuable contributions to future engineering in the areas of biomass conversion, sustainable energy and renewable resource development.

Project's Role in Center's Strategic Plan

The project is most directly tied to Thrust 3 in the Center's strategic plan and allows us to bring in new capabilities not possible through our US network of partners. The international experiences for students and the ability to work in large international teams will be an important component of the ERC strategic plan.

Fundamental Barriers and Methodologies

In establishing a large collaborative network, the first barrier was to get the partners to learn about the complementary expertise and how to integrate it into their projects. The US team members are actively working with each other, and the collaborations with the EU partners continue to grow. We have had changes in our collaborators in Denmark Prof. Claus Christensen moved from the Danish Technical University to Haldor Topsoe and then left Haldor Topsoe, Rafal Dunin Borokowski left DTU for Germany, but we have now established ties with Esben Taarning at HTAS and with Thomas Hansen at DTU. The research focus is on understanding of bimetallic catalysts and the application of in-situ spectroscopic techniques to liquid phase heterogeneously catalyzed reactions.

Foreign Collaborations

The major activities of the PIRE program involve collaborative research among the PIRE partners and EU collaborators, research visits by students and faculty from the PIRE partner institutions to our EU collaborators, our annual PIRE meeting and an annual PIRE summer school. Each of these is described in more detail below.

During 2012 we did not conduct our annual summer school in view of the International Congress of Catalysis (ICC) being held in Munich in July 2012. The ICC 2012 in July provided an opportunity for many of the PIRE faculty to meet with our international research partners. The meeting was attended by Hans Niemantsverdreit, Bert Weckhuysen, Krijn de Jong and Leon Lefferts from the Netherlands, by Malte Behrens from Germany and Dmitry Murzin from Finland. Attending from the US were Bob Davis, Matt Neurock and Abhaya Datye. No PIRE specific PI meeting was held but many informal interactions occurred. US co-PIs Robert Davis and Matt Neurock both presented keynote presentations at this meeting, providing an opportunity for showcasing the research done through the PIRE and ERC grants.

Faculty and Student Research Visits 2012

During summer 2012, graduate student Ronald Carrasquillo from the Dumesic group at Wisconsin spent 4 months in the laboratories of Professor Roberto Rinaldi and Professor Ferdi Schüth at the Max-Planck-Institut für Kohlenforschung. This research involved the use of advanced analytic instrumentation to characterize the samples of lignin-derived solvents that are being prepared at the University of Wisconsin. In addition, Ronald is working on new biomass processing methods that are being developed in Germany, such as ball-milling of biomass as a pre-treatment step prior to acid-catalyzed hydrolysis.

In June 2012, Prof. Laura Jarboe visited Dr Peter Duerre, Director of the institute for microbiology and biotechnology at Ulm University in Germany to establish a new collaboration. Amanda Anderson, undergraduate student at U-NM spent the summer at the Technical University of Eindhoven. She did research in the group of Prof. Hans Niemantsverdreit on nanoplasmonic structures based on Au/SiO_2. Derek Falcone from U-VA visited Haldor Toposoe during fall 2012 and worked with Esben Taarning.

Robert Davis visited the Netherlands in March 2012. He gave a plenary lecture at the 13th Netherlands' Catalysis and Chemistry Conference, Noordwijkerhout, The Netherlands, and he Visited the University of Twente, and presented a seminar.

Abhaya Datye visited the Fritz Haber Institute in February 2012 to attend a symposium on Electron Microscopy of Catalysts and also to discuss collaborative research. After this meeting, Prof. Datye also visited DTU in Denmark where he presented a seminar. A new collaboration was initiated as a result of the visit last year to Berlin with Gunther Rupprechter, formerly at the FHI in Berlin, and now a faculty member in Vienna. Prof. Datye visited Prof. Rupprechter's group in Vienna to present a seminar at the Technical University of Vienna. Finally, Prof. Datye also presented an invited lecture at the Topsoe Catalysis Forum in August 2012. This was the start of the celebration of the centennial year of Dr. Haldor Topsoe who turns 100 years in summer 2013. The workshop was devoted to the Electron Microscopy of Catalysts. Following this workshop, Prof. Datye also

attended a collaborative meeting of the Pd group organized by the Fritz Haber Institute in Berlin where he presented a talk on the research being done at UNM.

As evidence of the maturing of the PIRE program into a longer lasting partnership, we also find evidence of reciprocal hiring, where the participants gain employment in partner countries.

- Jean Phillipe Tessonier was hired in 2012 as a tenure track Assistant Professor at Iowa State University. He was formerly at the Fritz Haber Institute in Berlin and attended the PIRE 2010 summer school, after which he did a brief appointment at the University of Delaware.
- Edward Kunkes, formerly a PIRE PhD student at the University of Wisconsin, and then a post-doc at the Fritz Haber Institute in Berlin has now accepted a full time position at BASF at Ludwigshafen in Germany.

Another facet of a maturing partnership is the increasing number of reciprocal visits from our PIRE partners in the EU to the US research labs as described below:

- A Ph.D. student from the Danish Technical University, Christian Mårup Osmundsen, who is working in Denmark under the supervision of Esben Taarning, spent 3 months at the University of Wisconsin in Prof. Dumesic's group. Christian's Ph.D. research in Denmark involves the synthesis of new catalysts, such as Sn-beta-zeolite, for the conversion of sugars to chemicals. The recent work at Wisconsin has been exploring the conversion of dimethylfuran with ethylene to para-xylene. Thus, a research team comprised of Christian and another graduate student (Eric Wang) worked together to employ Christian's advanced catalyst synthesis skills along with the UW reaction kinetics studies to explore new catalysts for Diels-Alder synthesis reactions. A manuscript describing these results is being prepared for publication.

- Another Ph.D. student from the Åbo Akademi in Turku/Åbo, Finland, Alexei Kirili, who is working in Finland under the supervision of Dmitry Murzin, spent 3 months at UW. Alexei's research in Finland involves reaction kinetics studies of aqueous phase reforming reactions. While he was in Wisconsin, Alexei worked with graduate student Brandon O'Neill to study the reaction kinetics of formic acid conversion to H_2 and CO_2 over supported metal catalysts. (Formic acid is a biomass-derived molecule that can be used as a source of H_2.) The experimental data from these reaction kinetics measurements were then combined with results from density functional calculations in the Mavrikakis group at Wisconsin to formulate a micro-kinetic model of formic acid conversion combined with water-gas shift. A manuscript based on this work is under preparation for publication in the near future.

During the year, we also had reciprocal visits from our EU collaborators, Prof. Robert Schloegl visit UNM in February 2012 to discuss research collaborations and to present a seminar.

Achievements

We are pleased to announce that the proceedings from our 2010 PIRE summer school have

now been published. The publication was delayed due to the difficulties of publishing in an open access format, which was required by our collaborators from the Max Planck Society in Berlin. Hence, the publication was handled by the Max Planck Society and now includes open access in electronic form and also print on demand through a commercial source to make this volume widely accessible. We think this represents a wonderful opportunity for CBiRC faculty to present key concepts in the field of catalysts of Biomass in a format that will broadly accessible to researchers around the world.

Behrens, Malte and Abhaya Datye (eds.) *Catalysis for the Conversion of Biomass and Its Derivatives* Max Planck Research Library for the History and Development of Knowledge, Proceedings 2. Berlin: Edition Open Access (ISBN 978-3-8442-4282-9). 2013.

http://www.edition-open-access.de/proceedings/2/

Other Relevant Work

The PIRE partnership is unique in the field of catalysis since there are no large scale collaborations, to the best of our knowledge, between US and EU scientists.

Plans for the Next Year

We are planning our next summer school to be held in Turku, Finland in June 2013. Planning for the summer school started when several of the PIRE PIs met with Prof. Murzin at the ACS meeting in San Diego in April 2012. The summer school will provide an opportunity to further enhance the collaborations with the group at Turku. Further, it will be an opportunity for US students to learn about the extensive research on wood chemistry and the processing of wood based products that is the focus of work at Turku. During the coming year, we also will work with CBiRC faculty to allow a wider range of researchers (especially those involved in Thrusts 1 and 2) to develop collaborations within our partner countries. This will help broaden the focus of the research conducted by the PIRE to include biocatalysis and also allow CBiRC broadly to build on the collaborative partnerships developed by Thrust 3 through the PIRE program.

Expected Milestones and Deliverables

We already have 22 collaborative publications resulting from the PIRE project and we expect many more in the coming year.

Member Company Benefits

Member companies get early access to the research done by our EU partner institutions.

Commercialization / Curriculum Impacts

The international collaborations provide US faculty an opportunity to learn how the graduate student experience in Europe differs from the US approach. We see increasing emphasis on focused summer schools, and collaborative models that involve partnerships among schools in different countries. For example, the European graduate school on catalysis involves DTU, TU/Eindhoven and TU/Munich. The course materials being developed in those schools are widely disseminated and will serve as an excellent resource for our students within CBiRC.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: BioBased Foundry (*a sponsored project*)

Thrust: University Education Program and Innovation Ecosystem

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Peter L. Keeling	**02/29/2013**	**03/01/2012 to 02/28/2013**

ERC Team Members

Project Leader: Peter Keeling, Iowa State University

Other Faculty: Brent Shanks, Iowa State University

Statement of Project Goals

The project goal was to create and develop a mentorship program in Technology-Led Entrepreneurship related to the emerging BioBased sector. This was first created because of the Project Leaders experiences attending the NSF I-Corps Program in Stanford with Steve Blank. The Foundry was initially created within the framework of CBiRC's Innovation program in order to learn from other experiences in mentoring entrepreneurship in a university setting. A fundamental mission of the BioBased Foundry is to assist early stage entrepreneurs to realize the dream of founding a successful company. The students and faculty learn fundamental skills that all technologists and engineers should have when thinking about their new business concept. The Foundry is crafted around the "Business Model Canvas" used in the I-Corps Program and in essence has many of the elements taught by the NSF I-Corps Program.

Project's Role in Center's Strategic Plan

The BioBased Foundry was designed as an addition to the Universities efforts with a Proof of Concept Initiative. The Foundry contributes to the Center's strategic plan by developing an improved understanding of entrepreneurship and what it takes to be successful. The BioBased Foundry allows students and faculty from a variety of allied disciplines to understand the opportunities for developing biobased products. In addition, students and faculty in the Foundry gain explicit entrepreneurship experience, a background in the general issues related to production and processing of biorenewable resources and exposure to the economic and environmental realities of starting a successful new company.

Fundamental Barriers and Methodologies

The BioBased Foundry overcomes traditional barriers and misconceptions that entrepreneurship is difficult and that startups are likely to fail. The course impacts students and faculty by teaching them that entrepreneurship and forming a startup entity can be a rewarding intellectual challenge. The course impacts the Center by forming a foundation of understanding of the principles of starting a new business and how to work with potential customers and define a minimum viable product. The course introduces students and faculty to the concept of "getting out of the building" to meet with a customer base and understand product distribution channels. It is expected that this

will have a trickle-effect into the CBiRC faculty and the university as a whole. The course shows how new innovations and inventions leads to a startup company. This process is demonstrated to be fundamentally similar to the process of project evaluation in large companies. The course proceeds with a step-by-step review of how to put together professional business/project plans and presentations in order to position a startup entity or new project for sustained ongoing funding.

Achievements

In 2013, the Foundry was launched with four startup companies that were carefully selected by a university committee in charge of the ISU Proof of Concept Initiative. The companies included Gross Renewables, AccuGrain, WebChemi and SusTerea Biorenewables. For the Foundry, each company comprised 1 student, 1 industry mentor and 1 faculty (same model as I-Corps). All companies will complete the program by June 2013. The Foundry gained a lot from being part of an I-Corps grant which started in March, 2012 by enabling us to think through the content. All four startups were awarded seed funding from the base funds provided to the Foundry.

Other Relevant Work

There are many types and levels of entrepreneurship that are traditionally designed within the university business school curriculum. The BioBased Foundry focuses on Technology-Led Entrepreneurship and is a fundamental and vital departure from this traditional business-led realm.

Plans for the Next Year

The BioBased Foundry is planned to continue indefinitely within CBiRC as well as the university. In 2013, the Foundry is a vital adjunct to the Entrepreneurship course that has become a formal course requirement for the graduate minors in "Biorenewable Chemicals" as well as the "Biorenewable Resources and Technology".

Expected Milestones and Deliverables

The Foundry stimulates and nurtures an entrepreneurial spirit within CBiRC and ISU. We hope we can stimulate early stage successes and nurture these companies into securing funding.

Member Company Benefits

Industry members are inherently risk-averse. Yet they can gain from this, because startup entities are better able to deal with risk. Industry Members can become sources of funding and become strategic partners or mentors as well as customers. The industry members could eventually become owners of these startup entities.

Commercialization / Technology Transfer

Translational Research Grants are a superb source of funding for early stage startup entities. Creating startup entities is a fundamental metric of the Gen-3 ERC's.

Associated Project Abstracts

Provided in this section are abstracts for associated projects that are considered by CBiRC investigators to be integral to the center's research strategic plan or education strategic plan. In some cases, projects may have actually been awarded to non-ERC personnel, i.e., faculty and/or investigators outside the center, but partial funding was allocated to CBiRC faculty. To the extent practicable, current and proposed award year budget amounts for these projects as shown in Table 2, Volume I, reflect only the portion of such awards that is administered by the CBiRC faculty member's home department.

Further, in an effort to acknowledge other contributors/collaborators, CBiRC faculty members may have listed in their abstracts the names of the non-ERC PI/PD as Project Leader and non-ERC students and postdocs as team members. However, since these individuals were not directly involved in executing research funded by the center, or in carrying out ERC outreach activities, their demographic data were not collected, nor were they reported in Table 7 (ERC Personnel).

Thrust 1 — New Biocatalysts for Pathway Engineering

Biosynthesis of Alkamides - Experimental Modeling of a Modular Secondary Metabolic Pathway

Sponsor:	National Science Foundation
Project Leader:	Basil J. Nikolau, Iowa State University
Other Faculty:	Robert Minto, Indiana University – Purdue University at Indianapolis

This project is testing the feasibility of strategically applying high-throughput global profiling technologies to assess the expression of a complex genome and elucidate natural product biosynthetic pathways in a non-model species with an uncharacterized genome. Deciphering and defining the metabolic capability of the Echinacea genus to biosynthesize alkamides will test this strategy. Alkamides are a class of specialized metabolites that are biologically assembled via a modular metabolic pathway that may be an adaptation of amino acid and fatty acid metabolism. Expedient and informative experimental systems have been proposed that will combine metabolite profiling and metabolic flux studies, coupled with the transcriptomics analysis of alkamide biosynthetic tissues to identify genes and enzymes that assemble a diverse collage of alkamides. Specifically, studies of the alkamide pathway therefore offer the potential of discovering new metabolic processes and associated biocatalysts that generate novel combinations of chemical functionalities (fatty amides, alkyl chains with carbon-carbon double and triple bonds arranged with unusual regiochemistry), which have wide-ranging applications (e.g., lubrication and detergent industries). In addition, this proposal outlines a general methodology that should be broadly applicable to discovering how primary and specialized plant metabolism is juxtaposed and evolves to generate the physiochemical phenotypic differences among plant taxonomic groups. The proposed multilayered bio-prospecting offers the opportunity to browse the metabolic repertoire of an organism and, with system-wide knowledge

of the involved biochemical processes, should translate to the creation of novel bio-derived compounds relevant to the chemical industries, as well as strategies for pest- or disease resistance.

** This project is relevant to, and integrates across, both Thrusts 1 and 2.*

Characterization of Biocatalysts for Novel Production Platforms for Diverse Bi-functional Precursors of Polymers and Surfactants

Sponsor:	U.S. Department of Commerce ***(Translational Research)***
Project Leader:	Basil Nikolau, Iowa State University
Other Faculty:	Peter Keeling and Marna Yandeau-Nelson, Iowa State University

OmegaChea is developing fatty acid-based technology to address two potential market opportunities with new bio-based chemicals. The first of these is the polymer industry, which currently uses bi-functional monomers (i.e., a molecule with chemical functionalities at both ends) that are predominantly generated from non-sustainable petroleum feedstocks. The second market opportunity is the surfactant/lubricant industry, which is seeking bio-based bi-functional fatty acids (specifically branched chain fatty acids) that offer enhanced chemical-physical lubricity and tribological properties. Fatty acids provide attractive bio-based alternative "green" chemicals for both bio-polymers and surfactants or lubricants. The proposed i6-Green project will leverage the technology platform initially supported by CBiRC, and establish a startup entity (OmegaChea) to expand the KASIII IP several steps further. This will be accomplished by using select KASIII enzymes that can produce fatty acids with specific omega-end functionalities and can increase fatty acid production efficiency. OmegaChea will explore the scalability of these manufacturing fermentation processes. The project presented in this proposal is built upon the market studies and industry input garnered as a part of the NSF I-Corps grant awarded to explore the commercial potential of KASIII based technology to make bi-functional fatty acids. Through the I- Corps program, we have learned that branched chain acids, which perform better at lower temperatures, will be very attractive to numerous surfactant and lubricant companies, whereas the polymer industry would be attracted to hydroxylated fatty acids, because they are ideal monomers for bio-based polymers. The ultimate demonstration of optimized production of bi-functional fatty acids (new IP) in this project would allow our technology to proceed forward towards commercialization of such bio-based products.

Coenyzme B12-dependent 1,2-propanediol Degradation in *Salmonella*

Sponsor:	National Science Foundation
Project Leader:	Thomas Bobik, Iowa State University

The long-term goal of this research is to determine the specific functions of the 23 genes involved in 1,2-PD degradation, and to elucidate the molecular principles of the microcompartments involved in this process. The specific aims focus on the structure, function and assembly of the microcompartments involved in 1,2-PD degradation, and on B12 recycling. Microcompartments provide are contolled environment for optimization enzyme catalyzed reactions. A understanding of their functional and structural principles will provide information

helful for engineering designer microcompartments to enhance to production of renewable chemcials.

I-Corps: Novel Bio-Based Chemical Feedstocks for the Polymer Industry

Sponsor:	National Science Foundation ***(Translational Research)***
Project Leader:	Basil Nikolau, Iowa State University
Other Faculty:	Peter Keeling, Iowa State University

This Innovation Corps project will explore the potential of developing a marketable innovation based on the use of 3-ketoacyl ACP synthase III (KASIII) enzymes. KASIII catalyzes the first carbon-carbon forming reaction in fatty acid biosynthesis, and thus determines the chemical nature of the omega of the fatty acids produced. The proposed innovation is based on engineering of the biocatalyst, KASIII, and would result in the production of bi-functional molecules that can act as the monomer precursors for making bio-based plastics. This innovation will provide novel bi-functional, bio-based feedstocks to the emerging bio-renewable chemical industry for the production of novel "green" plastics and specialty chemicals. The proposed I-Corps team envisions a technology that will be initially targeted to the synthesis of hydroxy-fatty acids, but can be readily advanced upon to produce other bi-functional monomers (e.g., amino-fatty acids, which can be used to produce polyamides). If successful, this technology will lead to chemical products that can act as substitutes for petroleum-based chemical products, and be precursors for novel bio-based products.

Mechanistic and Structural Basis for Plant Metabolic Evolution

Sponsor:	Howard Hughes Medical Institute
Project Leader:	Joseph P. Noel, Salk Institute for Biological Studies

What shapes natural selection of specialized enzymes and metabolic pathways underlying the emergence and expansion of chemical diversity in living systems remains a fundamental yet largely unanswered question in evolutionary biology. For sessile organisms possessing the developmental and ecological complexity of plants, this adaptive process is especially critical to their survival. The chemical output of these metabolic pathways serve as key mediators of intra- and interspecies interactions resulting in speciation, survival and ecological homeostasis. Specifically, we probe the adaptive molecular changes that have occurred in plant specialized metabolism as these enzyme networks emerged and subsequently evolved from their ancestral roots in primary metabolism at the dawn of terrestrial plants. Our work examines the biosynthesis of plant natural products including isoprenoids, phenylpropanoids, polyketides and associated flavonoids and fatty acid-derived metabolites. Specialized metabolic pathways and their “chemical output” present us with a rich evolutionary record of where biosynthetic pathways, natural chemicals and biosynthetic enzymes have been (vestigial biochemical traits), what adaptive advantages these complex enzymatic systems hold in the present (emergent function), and ultimately where these pathways may be heading in the future (functional plasticity). Our decade-long study of these metabolic pathways has coalesced over the ensuing five years to answer a series of fundamental questions regarding the origin of specialized metabolism during land plant evolution. (i) Can one discern the phylogenetic routes through

which plant secondary metabolic enzymes evolved from their primary metabolic ancestors? (ii) What are the biophysical features inherited by these enzymes that give rise to evolvability and/or restrain such evolutionary processes? (iii) How was the evolutionary directionality maintained if at all before the emergence of the ultimate activities that provide obvious selective advantages? (iv) What role did catalytic promiscuity play in shaping the evolvability of these biosynthetic systems? Answering these questions not only will extend our understanding of the biochemical strategies that early land plants adopted in their adaptation to a myriad of terrestrial environments, but will also better shape our appreciation of mutability and the origins of new enzyme function in general.

Metabolomics: A Functional Genomics Tool for Deciphering Functions of Arabidopsis Genes in the Context of Metabolic and Regulatory Networks

Sponsor:	National Science Foundation
Project Leader:	Basil Nikolau, Iowa State University
Other Faculty:	Julie Dickerson, Philip Dixon, and Eve S. Wurtele, Iowa State University; Ruth Welti, Kansas State University; Lloyd Sumner, Noble Foundation; Sueng Rhee, Stanford University; Oliver Fiehn, University of California – Davis

Global profiling technologies enable comprehensive overview of the consequences of genetic alterations and can be used to annotate gene functions. However, the functions of over one-third of the annotated protein-coding genes of the *Arabidopsis* genome are still unknown, and the annotation of an even larger portion of the genome is not sufficiently accurate for unambiguous assignment function at the biochemical and physiological levels. This project builds on a prior pilot project that enabled a consortium of multidisciplinary collaborators to establish pipelines for generating metabolomics data streams and to integrate the outcomes with bioinformatics, computational, and database capabilities. Our goal is to develop novel capabilities that will enhance the research community's ability to formulate testable hypotheses concerning *Arabidopsis* gene function. The consortium has developed metabolomic platforms that together detect approximately 1,800 metabolites, of which 900 are chemically defined. The aims of the current project is to apply these established platforms to reveal changes in the metabolome associated with knockout mutations in 450 genes of unknown function and compare these to similar mutants in 50 genes of known function. To enhance the power of the metabolomics platforms, the consortium will begin analytical efforts to expand the chemical identity of the *Arabidopsis* metabolome. Finally, the consortium will disseminate these data via the multi-functional metabolomics database developed in the pilot project. Enhancement of this database and associated statistical and visualization toolsets will enable researchers to formulate testable computational models of the metabolic network of *Arabidopsis*. The successful completion of these goals and integration with other NSF-sponsored functional genomics and cyber infrastructure developments will generate transformational resources for ultimately modeling the complex metabolism of *Arabidopsis*.

** This project is relevant to, and integrates across, both Thrust 1 and University Education/ Outreach.*

SoLysis: A Start-up Focused on Novel Biocatalysts for the Production Platforms of Diverse Fatty Acid Products

Sponsor:	U.S. Department of Commerce ***(Translational Research)***
Project Leader:	Basil Nikolau, Iowa State University
Other Faculty:	Peter Keeling and Marna Yandeau-Nelson, Iowa State University

Fatty acids are biological molecules that have multiple applications in the industrial and nutritional economic sectors. These applications are dependent on the chemical-physical properties of the fatty acids dependent primarily on the carbon chain length of the alkyl-moiety of the fatty acid. SoLysis is developing acyl-ACP thioesterase biocatalytic technology for the fermentative production of nearly pure sources of individual fatty acids, ranging in chain lengths from 4-carbon (C4) to 18-carbon (C18) atoms. In this project, we will determine enzymatic activity and productivity for an additional 25 TEs by our established in vivo expression screening protocols (Jing et al., 2011). We will also seek additional funding to expand this effort so as to enable the characterization of the remaining TEs, which were initially computationally identified (360 in total). Moreover, since that computational screen was completed (~24-months ago) an additional ~350 TE sequences have been deposited in public databases, and if resources can be generated these "new" TEs will also be characterized. Initially these analyses will seek to identify chain length-specific TEs than can be used to fermentatively produce near pure (>80% purity) sources of a single fatty acid of different chain lengths (from C4 to C18). Even if chain length-specific TEs are not directly identified by these screens, the analysis of this larger number of TEs will allow us to deduce, predict and test the "rules" that govern both substrate specificity and fatty acid productivity. We will then have the ability to produce "designer" TEs for the biological production of fatty acids of different chain lengths, and potentially with different functionalities, which can then be used for specific applications.

Uncovering Novel Signaling Interactions in Plant Metabolic Networks

Sponsor:	National Science Foundation
Project Leader:	Eve S. Wurtele, Iowa State University
Other Faculty:	Ling Li, Iowa State University

This project investigates proteins of unknown function that regulate metabolism in plants. The work is important in that relatively little is known about the factors that influence carbon partitioning in cells, yet this understanding is crucial to acheivement of high yields of desired compounds. The genes we identify in this study might function directly in yeast, or have a homolog in this organism. Furthermore, the mechanisms of metabolilc regulation may be common across multiple organisms.

** This project is relevant to, and integrates across, both Thrust 1 and University Education/ Outreach.*

Thrust 2 — Microbial Metabolic Engineering

A Native Pathway for the Production of N-butanol in *Eschericia Coli*: A New Paradigm for Synthetic Biology

Sponsor:	National Science Foundation
Project Leader:	Ramon Gonzalez, W. M. Rice University

Interest in the use of advanced biofuels, such as n-butanol and other higher-chain linear alcohols, has rapidly developed because they offer several advantages compared to ethanol, including less hygroscopicity and volatility, higher energy density and compatibility with current infrastructure for storage, distribution and usage. Among linear alcohols currently considered as advanced biofuels, n-butanol is the only one found in nature as a major fermentation product. The ability to synthesize n-butanol is considered to be an exclusive feature of clostridial species. *Clostridia* are spore formers, obligate anaerobes that grow at slow rates, have complex nutritional requirements and produce n-butanol along with a mixture of other products including acetone, ethanol, butyrate, and acetate. The lack of efficient genetic tools to manipulate clostridia, along with their complex metabolism, hinders metabolic engineering efforts that could lead to the improvement of n-butanol yield, titer, and productivity. In an effort to overcome the aforementioned issues, the genes that enable the synthesis of n-butanol in native producers like clostridia have been imported into industrial organisms that are genetically and metabolically tractable such as *E. coli*, *Saccharomyces cerevisiae*, *Pseudomonas putida*, *Bacillus subtilis*, *Lactococcus lactis*, and *Lactobacillus* species. All efforts to date have been based on what we refer to in this project as heterologous metabolic engineering (HeME): that is, transplanting genes/pathways of (primarily) clostridial origin to hosts otherwise not able to produce butanol (e.g. *E. coli*, *S. cerevisiae*). HeME-based approaches have been used to engineer biofuel production in the past and are currently viewed as the strategy of choice when the host organism does not possess the desired metabolic function. However, in the case of n-butanol and other linear n-alcohols, HeME approaches have faced significant hurdles. For example, after several years of strain development and optimization, organisms engineered for the production of n-butanol synthesize this alcohol at low flux and still require the supplementation of the medium with rich nutrients. We hypothesize that the use of a heterologous metabolic engineering approach represents the main issue accounting for the limited success of the aforementioned studies, as it relies on transferring a heterologous pathway that might not be compatible with the host, thus compromising its functionality.

The intellectual merit of the work relates to addressing the aforementioned limitations by developing an alternative strategy that focuses on the identification and harnessing of native *E. coli* enzymes/pathways that could act as surrogates of the heterologous n-butanol-synthesis pathway and hence mediate the synthesis of a non-native product in the absence of foreign genes. Since no exogenous gene is recruited to establish the otherwise foreign pathway, we have termed this approach “homologous metabolic engineering” (HoME). The overall goal of this project is to identify, characterize and harness native biosynthetic pathways for the efficient production of n-butanol in *E. coli*, thus establishing a new paradigm for the application of synthetic biology to the production of advanced biofuels. The specific objectives of the work are: i) identify native *E. coli* genes encoding enzymes that can catalyze the reaction steps comprising the clostridial

butanol pathway; ii) in vivo assembly and functional characterization of a native butanol pathway in *E. coli*; iii) improve the efficiency of the native n-butanol pathway; and iv) system-wide characterization of wild-type and engineered strains.

The broader impacts of the project are numerous. The establishment of HoME as a new paradigm for metabolic engineering and synthetic biology would lead to exploiting the multi-potent capabilities of native hosts via engineering of functional differentiation. By enabling the production of n-butanol through a homologous pathway, this proposal will contribute to the creation of fundamentally new approaches that could enable efficient production of second-generation biofuels in many industrial organisms. Based on these advances, efficient and economically viable chemical and biofuel industries can be developed that will make possible energy independence and climate protection. This project will also educate our society in the scientific and engineering challenges and opportunities on the road to a sustainable energy future. We will capitalize on our collaborations with the Houston Harmony Science Academy to train middle and high schools students in the field of alternative energy. This school serves predominantly minority populations, and thus, we will address the national need, and challenge, of increasing their participation in science and engineering.

A Robust Platform for Reconstituting and Engineering Iterative Megasynthases

Sponsor:	National Institutes of Health
Project Leader:	Yi Tang, University of California – Los Angeles
Other Faculty:	Nancy A. Da Silva, University of California – Irvine

Nature uses an amazing array of enzymes to make small molecule natural products. Among the most interesting but least understood enzymes making these compounds are the iterative polyketide synthases (IPKSs) found in filamentous fungi. In contrast to the well-studied bacterial type I PKSs that operate in an assembly-line fashion, IPKSs are megasynthases that function iteratively by using a single set of catalytic domains repeatedly in different combinations to produce structurally diverse fungal metabolites. Bioinformatics analysis of the genomes of recently sequenced fungal species revealed that each genome contains a large number of genes encoding IPKSs. The total numbers of IPKSs significantly outnumber the known polyketides and polyketide-nonribosomal peptides isolated from these species, suggesting that a majority of biosynthetic genes are silent in these fungi under cultivating conditions. This in turn suggests that the fungal species may have untapped potential to synthesize a much large number of natural products. Furthermore, analysis and engineering of IPKSs have been hampered by inability to obtain sufficient amounts of the functional purified megasynthase from either the native fungal host or heterologous *Aspergillus* hosts. As a result, the programming that governs metabolite assembly by IPKSs is not understood. Key aspects that remain to be elucidated include: 1) the catalytic and structural roles of each domain in the megasynthase; 2) substrate specificities of the catalytic domains and their tolerance to perturbation in egasynthase functions; and 3) factors governing the choice of different combinations of catalytic domains during each iteration of catalysis. The objective of this proposal is to develop the genetically superior *Saccharomyces cerevisiae* as a heterologous host for reconstitution, analysis and engineering of IPKSs, especially the enigmatic highly-reducing IPKS, such as LovB associated with Lovastatin biosynthesis. We have accumulated a significant body of preliminary data to demonstrate that *S. cerevisiae* is a highly robust host for expressing these megasynthases in functional forms, and

can facilitate the production of polyketide products both in vivo and in vitro with purified enzymes. The following specific aims will be pursued: 1) Engineer and optimize *S. cerevisiae* towards producing fungal metabolites and megasynthases; 2) Reconstitution of fungal megasynthases in *S. cerevisiae*; 3) Biochemical analysis of fungal PKS using *S. cerevisiae*; and 4) Genome mining of filamentous fungi using *S. cerevisiae* as a host.

** This project is relevant to, and integrates across, both Thrusts 1 and 2.*

Biological Utilization of Thermolytic Substrates by Bacteria and Microalgae

Sponsor:	National Science Foundation
Project Leader:	Laura Jarboe, Iowa State University

The overall goal of this work is to use microorganisms to produce biofuels from biomass. This project is distinctive in that we aim to produce sugar substrates for these microorganisms by fast pyrolysis of biomass, instead of hydrolysis or gasification. Economical analysis has shown that this is a viable and competitive method for producing biofuels. We will produce two model biofuels or biofuel feedstock, ethanol and lipids, by two model microorganisms, *Escherichia coli* and *Chlamydomonas reinhardtii*. Fermentation of the fast pyrolysis product, also known as bio-oil or thermolytic substrates, is limited by the fact that in addition to useful substrates, it also contains many inhibitory "contaminant" compounds, including furans and phenols. At Iowa State University, we have a pyrolysis method that enables the collection of bio-oil in distinct fractions. This fractionation changes the distribution of substrates and of these inhibitory contaminant compounds. This decreases but does not eliminate the problem of contaminant toxicity. Addressing this toxicity is the main focus of this project.

We aim to increase the tolerance of our two microorganisms to these inhibitory contaminants, so that the need for additional purification processing of the pyrolysis product is reduced. We will use metabolic evolution to increase the robustness of our microorganisms to the contaminants, largely because the bio-oil is complex and the mechanism of the inhibition is not known. The outcomes of this work include microorganisms that can utilize raw thermolytic substrates for production of biofuels and a roadmap for engineering other microorganisms for this ability.

Collaborative Research: Metabolic Engineering of Terpenoid Indole Alkaloids Using Transcriptional Regulators in *C. Roseus* Hairy Roots

Sponsor:	National Science Foundation
Project Leader:	Jacqueline V. Shanks, Iowa State University

Plants produce large numbers of chemicals that are useful as drugs for treating a variety of medical conditions. Unfortunately, these chemicals are often produced by plants in only trace amounts. As a result, large quantities of plant material must often be used to produce small amounts of drugs, making these drugs very expensive. An example of this problem is provided by a class of compounds produced by Catharanthus roseus (periwinkle), known as terpenoid indole alkaloids. Terpenoid indole alkaloids include chemicals such as vincristine and vinblastine, which are used to treat certain types of cancer, and ajmalicine, which is used to treat hypertension. As periwinkle produces these chemicals in only trace amounts, they can cost over

one million dollars per pound. To reduce the costs of these drugs, it is critical to develop varieties of periwinkle that produce these drugs in higher amounts. Towards that end, the effects of increasing expression of the biosynthetic genes that are responsible for production of these drugs will be investigated. A two-fold strategy for increasing expression of these biosynthetic genes will be pursued. Part one of this strategy is to test the effects of reducing expression of repressor genes that decrease expression of the biosynthetic genes. Part two of this strategy is to decrease expression of the repressor genes while simultaneously increasing expression of activator genes that increase expression of the biosynthetic genes. The hypothesis being tested is that, by decreasing expression of repressors and increasing expression of activators, it should be possible to achieve a significant increase in expression of the biosynthetic genes. This increase in expression of the biosynthetic genes should then lead to increased production of terpenoid indole alkaloids, allowing those terpenoid indole alkaloids that are useful as drugs to be produced at a lower cost. Previously uncharacterized chemicals that accumulate in response to alterations in expression of the biosynthetic genes will also be analyzed. Characterization of these chemicals will both provide valuable information regarding the biochemical pathways by which terpenoid indole alkaloids are produced, as well as potentially providing information regarding additional chemicals that may be tested for use as drugs.

Efficient Synthesis of Hydrocarbons Using an Engineered Reversal of the β-Oxidation Cycle: A New Paradigm for the Production of Advanced Biofuels

Sponsor:	National Science Foundation
Project Leader:	Ramon Gonzalez, W. M. Rice University

In recent years much effort has been devoted to the production of biofuels, such as ethanol, butanol and higher-chain alcohols, via microbial fermentation of sugars and other biomass constituents. Interest in the use of advanced biofuels, such as hydrocarbons, has rapidly developed because they offer several advantages compared to alcohols, including less hygroscopicity and volatility, higher energy density and compatibility with current infrastructure for storage, distribution and usage.

Biosynthetic pathways leading to the synthesis of saturated (alkanes) and unsaturated (alkenes) hydrocarbons have been reported in the literature for decades, but not until recently were the corresponding biosynthetic genes identified. The "head-to-head" condensation of acyl-CoA thioesters has been proposed as the primary pathway to generate long-chain alkenes. On the other hand, alkanes are synthesized through a two-step pathway that involves conversion of acyl-CoA thioesters to fatty aldehydes, which are then decarbonylated to alkanes. To date, the fatty acid biosynthesis pathway has been used as the exclusive means to generate the aforementioned acyl-CoA thioesters required for the synthesis of hydrocarbons. However, the operation of this pathway is not efficient because it consumes ATP in the synthesis of malonyl-ACP, which is the donor of two-carbon units for chain elongation. As a consequence, the ATP yield associated with the production of hydrocarbon through the fatty acid synthesis pathway is very low. This, in turn, greatly limits cell growth and hydrocarbon production.

In this study, we use a functional reversal of the β-oxidation cycle, which was recently engineered in our laboratory, as a metabolic platform for the synthesis of hydrocarbons. Unlike the fatty acid biosynthesis pathway, the reversal of the β-oxidation cycle operates with

coenzyme-A (CoA) thioester intermediates and uses acetyl-CoA directly for acyl-chain elongation (rather than first requiring ATP-dependent activation to malonyl-CoA). These characteristics enable product synthesis at maximum carbon and energy efficiency. We have already demonstrated the superior nature of this metabolic platform by producing higher-chain linear n-alcohols ($C \geq 4$) and extracellular long-chain fatty acids ($C > 10$) at yields and titers an order of magnitude higher than previously reported.

The intellectual merit of this project relates to the efficient synthesis of hydrocarbons using an engineered reversal of the β-oxidation cycle, which in turn will establish a new paradigm for the production of advanced biofuels. Four specific objectives are proposed to accomplish this: i) Engineer a functional reversal of the β-oxidation cycle with a minimal set of enzymes; ii) engineer pathways for the synthesis of alkanes and alkenes (olefins) from acyl-CoA intermediates generated in the functional reversal of the β-oxidation cycle; iii) improve the efficiency of the engineered reversal of the β-oxidation cycle during the synthesis of hydrocarbons; and iv) system-wide characterization of wild-type and engineered strains.

The broader impacts of the work are numerous. The ubiquitous nature of β-oxidation enzymes should enable the combinatorial synthesis of non-native products in industrial organisms with a minimum number of foreign genes, an approach that would ensure the efficient functioning of the engineered pathways. By enabling the production of hydrocarbons through a functional reversal of the β-oxidation cycle, this proposal will contribute to the creation of fundamentally new approaches that could enable efficient production of second-generation biofuels. Based on these advances, efficient and economically viable chemical and biofuel industries can be developed that will make possible energy independence and climate protection. This proposal will also educate our society in the scientific and engineering challenges and opportunities on the road to a sustainable energy future. We will capitalize on our collaborations with the Houston Harmony Science Academy to train middle and high schools students in the field of alternative energy. This school serves predominantly minority populations, and thus we will address the national need, and challenge, of increasing their participation in science and engineering.

EFRI-HyBi: Bioengineering a System for the Direct Production of Biological Hydrocarbons for Biofuels

Sponsor:	National Science Foundation
Project Leader:	Jacqueline V. Shanks, Iowa State University
Other Faculty:	Basil Nikolau, D. Raj Raman, Govind Nadathur, and Gordon Wolfe, Iowa State University

This project will develop new bio-engineering technology for transforming the current liquid fuel industry from using fossil-carbon feedstocks to using biorenewable feedstocks that are at the chemical level identical to gasoline and diesel fuels, namely biologically-generated hydrocarbons. The engineering system we envision is a photosynthetic-based organism that will have the bio-engineered ability to chemically-reduce atmospheric CO2 to simple hydrocarbons (e.g., n-alkanes and n-alkenes), using sunlight as the source of renewable energy. Such metabolic conversions are known to occur in discreet places in the biosphere, e.g., the epidermis of plants and insects, and as a carbon/energy-storage mechanism by certain algae. Our goal is to conduct multidisciplinary studies that will identify the mechanisms and genetic elements that encode the

biocatalyst(s) that generate these hydrocarbons in biological systems. We will explore the use these isolated genetic elements to establish to bio-engineer crops or bioengineer photosynthetic microbes as the production platform to realize the vision of producing a biological hydrocarbon based fuel. The proposed research will for the first time lead to fundamental knowledge concerning the structure and mechanism of the biocatalyst that generates biological hydrocarbons. And, the efficient use of this novel biocatalyst in a production biological host will require the optimization of bioengineering principles so as to proficiently integrate the biocatalyst into a pre-existing metabolic network without compromising the biological competence of the host. These later optimizations will integrate concepts of biological control principles with engineering proficiencies. This project brings together a collaborative team of biologists and engineers to demonstrate a paradigm of how fundamental molecular biological research can be integrated with disciplines of engineering to generate new bio-engineered organisms that can be used as a sustainable production platform to meet the global demands for new liquid biofuels. An REU program and an international collaborative are venues for training undergraduate students and graduate students/postdoctoral associates, respectively.

* *This project is relevant to, and integrates across, both Thrusts 1 and 2.*

Energy Efficient Cultivation of Microalgae and Simultaneous Separation of Products using a Novel Taylor Vortex Reactor-Separator

Sponsor:	ConocoPhillips Company
Project Leader:	Dennis Vigil, Iowa State University
Other Faculty:	Jacqueline V. Shanks, Iowa State University

Large-scale production of biofuel from microalgae requires not only the development of elite fuel-producing microorganisms, but it also requires novel process engineering approaches that (1) significantly accelerate the rate of photosynthesis and that (2) provide energy-efficient methods for harvesting and separating algae and biofuel products. One of the most important factors for increasing the algal photosynthesis rate is high-frequency periodic exposure of cells to light and dark regions of the suspending fluid, and such high-frequency periodic exposure cannot be achieved in pond systems or conventional photobioreactors. Furthermore, since algae ponds and photobioreactors do not provide any natural mechanisms for harvesting mature algae and recovering biofuel products, expensive downstream separation processes must be employed. The purpose of the proposed work is to develop a novel continuous-flow reactor-separator that exploits a highly efficient self-organized flow pattern (Taylor vortices) to increase the rate of photosynthesis by rapidly shuttling microorganisms between light and dark regions of the reactor while simultaneously achieving centrifugal product separation. The proposed reactor-separator offers the additional advantages of good contacting of gas and liquid phases, low probability of rupturing algal cells, and nearly ideal plug flow behavior. A preliminary analysis suggests that this novel algal reactor-separator has the potential to produce biofuel at an energy cost that is approximately 8% of the energy produced, compared to an estimated 30% for conventional photobioreactors, which do not achieve product separation. The development and analysis of the Taylor vortex algae reactor-separator will be accomplished by building a bench scale reactor-separator prototype; carrying out an experimental program to determine how geometric and operating variables influence photosynthesis rate and reactorseparator operating costs; and developing computational models suitable for evaluating the scaleup potential of the device.

Engineering Yeast Consortia for Surface-Display of Complex Cellulosome Structure: A Consolidated Bioprocessing Approach from Cellulosic Biomass to Ethanol

Sponsor:	National Science Foundation
Project Leader:	Wilfred Chen, University of Delaware
Other Faculty:	Rachel Chen, Georgia Tech.; Nancy A. Da Silva, University of California – Irvine

According to the new Energy Policy Act, a billion gallons of renewable fuel must be produced by 2012 with most of that produced as biofuel using renewable biomass. In particular, bioethanol from renewable sources provides an attractive form of alternative energy. The primary obstacle impeding the more widespread production of energy from biomass is the absence of a low-cost technology for overcoming the recalcitrance of these materials. It has been shown that the overall cost can be significantly reduced using a one-step "consolidated" bioprocessing (CBP) of lignocellulose to bioethanol, where cellulase production, cellulose hydrolysis and sugar fermentation can be mediated by a single microorganism or microbial consortium. Cellulosomes are self-assembled multi-enzyme complexes presented on the anaerobes' cell surface and are dedicated to cellulose depolymerization. This self-assembled system brings multiple enzymes in close proximity to the substrate, and provides a structure that ensures high local concentration and the correct ratio and orders of the enzymes, thereby increasing cellulose hydrolysis synergy up to 50-fold. The objective of this project is to develop a synthetic yeast consortium for direct fermentation of cellulose to ethanol with productivity, yield, and final concentration close to that from glucose fermentation. The specific objectives are: 1) Construct a yeast consortium for surface assembly of a mini-cellulosome structure consisting of three cellulases and demonstrate the feasibility of using the consortium for direct ethanol production from cellulose; 2) construction of yeast strains for surface-display of the anchoring scaffoldin, strains for secreting the adaptor scaffoldin, and strains for secreting the dockerin-tagged cellulases; and 3) demonstrate the feasibility of the constructed yeast consortium to display the complex cellulosome and the ability for direct fermentation of cellulose to ethanol. The engineering strategy proposed emphasizes the efficiency of hydrolysis and synergy among cellulases, rather than focusing on the amount of enzymes produced or used.

Evaluate and Identify Metabolic Control Points Determining Assimilate Partitioning in Developing Seed

Sponsor:	Pioneer Hi-Bred International
Project Leader:	Jacqueline V. Shanks, Iowa State University

The factors that control resource partitioning in soybeans are poorly understood but are of great economic importance due to a strong negative correlation between oil and protein contents. Here we propose to use soy somatic embryos as models for the analysis of resource partitioning using metabolic flux analysis. Should the embryo system prove to be a suitable model (show reproducible changes in pathway fluxes in response to experimental perturbations), we propose to probe for flux control points in a series of experiments designed to influence assimilate partitioning using nutritional, physical and transgenic perturbations. Potential control points,

identified during the initial phases of experimentation, will be targeted using transgenic techniques in subsequent experimentation.

Generation of Biofuels from Abundant Non-Digestible Oilseed Components

Sponsor:	National Science Foundation
Project Leader:	Ka-Yiu San, W. M. Rice University
Other Faculty:	George N. Bennett, W. M. Rice University

The production of biofuels from renewable resources is currently an area national interest. In particular, butanol isomers have gained interest as an alternative fuel due to their superior fuel properties relative to ethanol. This research will use metabolic engineering approaches to enable the fermentation of the non-digestible, soluble carbohydrate fraction isolated from many common oilseeds such as soybean, cottonseed, and sunflower seed, to butanol. Many of these materials are byproducts of biodiesel or bioethanol production. For example, large amounts of galactose-rich soluble carbohydrates, constituting approximately 10% of oil seed weight, represent a sustainable yet underutilized feedstock for biofuel. Towards this end, the hydrolysis of the galactose-rich oligosaccharides and subsequent fermentation to 2-butanol by engineered *E. coli* will be studied and optimized. Furthermore, natural and engineered strains of *Clostridia* may also be able make effective use of this feedstock. Genetic modules for engineered strains of *Clostridia* will be developed that enable the efficient uptake and metabolism of galactose-rich oligosaccharides and their fermentation to 1-butanol.

Genetic and Environmental Factors Driving *E. coli* Attachment to Particles in Streams

Sponsor:	National Science Foundation
Project Leader:	Michelle Soupir, Iowa State University
Other Faculty:	Laura Jarboe, Iowa State University

Harmful microorganisms are the leading cause of impairments in the 300,000 miles of rivers and shorelines and 5 million acres of lakes that do not meet U.S. water quality standards. Modeling microbial transport in surface streams requires numerous assumptions because of the lack of understanding about the mechanisms driving microbial attachment to particles. Attachment to particles may be influenced by a genetic predisposition of bacteria to express outer membrane features that facilitate attachment, by physiochemical characteristics of organic particles or sediments, by water chemistry or environmental conditions, or some combination of these factors. The goal of the project is to improve our understanding of the genetic and environmental factors influencing *E. coli* attachment to organic particles and stream sediments. The objectives of the project are to (1) evaluate the attachment of *E. coli* to model stream particles under environmentally relevant conditions and determine the relative contribution of surface proteins and EPS to this attachment; (2) determine the effect of outer membrane proteins and EPS on attachment and identify the genes enabling expression of these features; (3) compare attachment and detachment behavior of *E. coli* with a demonstrated propensity for attachment; and (4) assess the mechanisms of *E. coli* attachment to actual stream particulates by comparison with attachment to model particulates. We will identify genetically encoded factors that mediate *E. coli* attachment to environmental particles. All findings will be confirmed by gene deletion

experiments to verify the role of proposed attachment-mediating genes on attachment under environmentally relevant conditions. Experiments will identify particle characteristics enabling *E. coli* attachment and environmental changes encouraging detachment of *E. coli* from environmental particles. The knowledge gained about the interactions between *E. coli* and particles is needed to improve understanding and modeling of microbial environmental fate and transport processes, which are important for the protection of high quality water supplies and identifying conditions when a risk to public health may be present. Understanding how pathogens flow through streams is important for predicting when public health might be at risk. Pathogens attach to particles when they move through waters, but it is unknown if this is because of the genetics of the bacteria, properties of the particle, or the environment surrounding the microorganism. This project will explore the many factors influencing the interactions between pathogens and particles in freshwater streams. This new knowledge will improve models of microorganism fate and transport, which are frequently used in watershed-scale, water quality assessments. We will also develop discovery-based learning modules for underserved minority middle school students and provide educational opportunities for several graduate and undergraduate students.

Lignocellulosic Biomass Conversion to Infrastructure Compatible Fuel, Products and Power

Sponsor:	U.S. Department of Agriculture ***(Translational Research)***
Project Leaders:	E. Elangovan, M. Karanjikar, S. Spatari, P. Vadlani, and W. Rooney, Ceramatec, Inc.
Other Faculty:	Ka-Yui San, W. M. Rice University

This USDA National Institute of Food and Agriculture (NIFA) project is an R&D collaboration involving Ceramatec, Inc. (prime recipient), Rice University and Texas A&M University and will convert lignocellulosic biomass to infrastructure-compatible renewable diesel, biolubricants, animal feed and biopower. New hybrids of energy sorghum will be developed, and other biomass resources include switchgrass and forestry residues. The biomass will be converted to hydrocarbons (molecules that are just like petroleum based hydrocarbons but derived from biomass) using innovative pretreatment, fermentation and electrochemical technologies. These hydrocarbons will be finished into premium synthetic bio-lubricants and biofuels via commercial petroleum refinery processes. A life cycle analysis will include energy efficiency impacts and assessment of impacts on rural development.

Objectives: The overall objective of the proposed project is to effectively integrate economic biomass production and conversion technologies to produce drop-in fuels, chemicals and power. The various sub-objectives are: i. Feedstock Development (Upstream) 1. Develop new energy sorghum hybrids with amenable traits for conversion. 2. Maximize the yield of energy sorghum while minimizing the agronomic inputs to keep the feedstock costs below $50 per Ton at the farm gate. 3. Identify the most economical solution for biomass logistics. 4. Integrate feedstock development with biomass logistics to supply lowest cost (<$60/Ton at refinery gate) feedstock to a biorefinery in the desired form and quantities. 5. Optimize regional supply of multiple feedstocks (Energy sorghum, switchgrass and forestry residue) to ensure year round availability of biomass for uninterrupted production of biofuels and chemicals. ii. Feedstock Utilization (Downstream) 1. Integrate biomass pretreatment, fractionation and hydrolysis with feedstock on

the upstream and conversion technologies downstream. 2. Develop the bioprocess for biomass conversion to free fatty acids (FFA). 3. Develop FFA conversion to hydrocarbons process via electro-catalytic process. 4. Convert the hydrocarbons to certified diesel via Chevron's proprietary mild hydrofinishing process and into high quality biolubricants (Group III) via Chevron's Isodewaxing process. 5. Integrate lignin conversion to power to improve process economics of the biorefinery. iii. Analysis Development (Integration) 1. Demonstrate the techno-economic feasibility for integrated fuels, chemicals and power generation from biomass at distributed co-operative scale (~10,000 acres of land) 2. Assess the rural socio-economic impact of the prospective commercialization using Jobs and Economic Development Impact (JEDI) models and the potential for displacing fossil fuels. 3. Develop sustainability metrics using LCA and DayCent for the proposed pathway. 4. Develop a hierarchical commercialization scheme, which counters risk of early adoption by high margin products (premium synthetic bio lubricants) 5. Evaluate the overall sustainability of the proposed pathway using spatially-explicit risk-uncertainty approaches, and integrate these with financial risk, LCA and JEDI.

Approach: The project will focus upon developing new energy-sorghum hybrids that are more amenable to conversion. Low cost logistics using novel size reduction and densification will be addressed. The project will further focus on producing diesel and premium synthetic biolubes from biomass via a combined low cost and hydrogen-independent bio-electrochemical pathway. Renewable power will be generated onsite to improve the overall economics and provide low carbon process energy. Detailed sustainability analysis will be used as decision support tools for value chain integration. Detailed rural benefit analysis will be performed using jobs and economic development impact (JEDI) model. Genotypes with high yield potential and desirable composition will be hybridized to standard seed parent sorghum lines to assess the potential to produce the hybrid and the effect that hybridization will have on productivity and composition. Hybrids will be produced using standard seed parents available in our sorghum-breeding program in a winter nursery in Puerto Rico between year 1 and year 2 of the research plan. If needed, additional hybrids will be generated between year 2 and year 3 for evaluation in year 3. In the biomass sorghum development program, improvement will focus primarily on pollinator parents; existing seed parents are sufficient for producing biomass sorghum hybrids. Pollinator parent line development will use a standard pedigree breeding approach. Biomass storage experiments will be performed via standard wet and dry techniques. The biomass composition changes will be analyzed using NREL method. Biomass size reduction and densification will be performed via collision mill and cubing machine respectively. The fractionation and hydrolysis will be performed via novel acid hydrolysis followed by detoxification technology. The biomass sugars and hydrolysate will be converted to free fatty acids via advanced bioprocessing. The free fatty acids will be converted to hydrocarbons via electro-catalytic method. The various analytical techniques will include HPLC, GC and GC-MS. Overall mass and energy balances will be performed periodically to interpret the results. The measured data will be mapped against a set of milestones to measure success.

Mass Spectrometric Imaging of Plant Metabolites

Sponsor:	U.S. Department of Energy
Project Leader:	Basil J. Nikolau, Iowa State University
Other Faculty:	Mei Hong, Robert Houk, Young-Jin Lee, Nicola Pohl, and Edward Yeung, Iowa State University

This project is developing mass spectrometric imaging techniques to map metabolite distributions within tissues, and eventually among individual cells. Mass spectrometry not only allows positive identification of the many metabolites but can also reveal the substrates and precursors involved in each metabolic pathway. Such information will provide unprecedented details on the distribution of metabolites from cell to cell, cooperative and antagonistic effects among the metabolites, and environmental influences on metabolism. Such details will ultimately lead to a predictive understanding of the mechanisms that multi-cellular organisms use to regulate metabolic processes. In the current work, we are focusing on the lipids of Arabidopsis. By studying the diversity of the lipids, we hope to gain detailed insight into their biosynthesis as a function of genetics, tissue type, development, and environment. In analogy to matrix- assisted laser desorption ionization (MALDI), a laser beam will be used to interrogate sequentially micrometer areas of a plant by vaporizing the surface contents of the tissue into a mass spectrometer. Rastering of the laser beam over the tissue will produce a laterally resolved image of the various substances within different structures of the plant. Repeated vaporization at the same focused point of a plant structure will produce a depth profile of the components. We plan to generate ions directly from the plant tissue by designing novel additives as pseudomatrixes. By minimizing sample preparation, compositional integrity and spatial resolution of the analysis will be guaranteed. Identification of the metabolites will be aided by new strategies in carbohydrate sequencing and in 2D-NMR.

* *This project is relevant to, and integrates across, both Thrusts 2 and 3.*

MRI: Acquisition of a Tandem (IT-TOF) Mass Spectrometer System for Biological Research and Application

Sponsor:	National Science Foundation
Project Leader:	Ka-Yiu San, W. M. Rice University
Other Faculty:	George N. Bennett, W. M. Rice University

The goal of this proposal is to establish a central mass spectrometry facility for biological research and education. To achieve this goal, a multidisciplinary research team has been assembled. The facility will provide much needed quantitative and analytical instrumentation for a number of biological projects. They propose to equip the facility with an ion trap time of flight (2D-nano-LC-IT-TOF) mass spectrometer.

Small Business ERC Collaborative Opportunity to Develop a Biomass Conversion to Fatty Acids Platform

Sponsor:	National Science Foundation ***(Translational Research)***
Project Leader:	Ka-Yiu San, W. M. Rice University

Research and development work at the NSF Engineering Research Center for Biorenewable Chemicals (CBiRC) has identified a bio-process for conversion of sugars to fatty acids as a platform technology. The fatty acid synthesis pathway can serve as an extremely versatile platform for the production of a very diverse class of chemicals. Due to rising demand and constrained supply, fatty acids are currently produced from palm or coconut oils. Due to rising demand and constrained supply, fatty acid prices have quadrupled in the last decade and are projected to rise further. The proposed effort will enable a broad platform for commercialization of various biomass-derived fatty acids and their derived products. Such a platform will cater to many industry sectors including consumer products, nutrition, personal care and polymers based upon carbon chain length of the fatty acids. The proposed effort will focus upon commercialization of C14 and C16 fatty acids. Technology Holding, LLC (small business) and Rice University (CBiRC) have collaborated to develop and commercialize the patent-protected biomass conversion to fatty acids and fatty acid derivatives platform. The proposed process utilizes patent-pending metabolically engineered *E. coli* culture to convert C6 sugars from biomass to fatty acids. The metabolically engineered strains have been shown to exhibit extremely promising performance — these strains can produce fatty acids at a fatty acids/glucose yield close to the maximum theoretical value and at good production rate. The goal of the proposed collaboration is to translate the existing ERC platform research into the marketplace by developing a hierarchy of fatty acid and derivative products synthesis based upon yield and market price. The commercialization effort will focus upon: (1) Demonstrating the intellectual property protected fatty acid based platform technology via scale-up; (2) Rapidly transitioning the fatty acid based platform technology to the marketplace aligned with the translational goal of CBiRC; and (3) Creating an economically viable product portfolio from the platform.

Use of Systems Biology Approaches to Develop Advanced Biofuel-Synthesizing Cyanobacterial Strains

Sponsor:	U.S. Department of Energy
Project Leader:	Himadri Pakrashi, Washington University
Other Faculty:	Costas D. Maranas, Pennsylvania State University

The objective of this proposal is to utilize a systems biology approach to develop a knowledgebase of the metabolic and regulatory networks involved in the photobiological production of advanced biofuels and/or their chemical precursors by cyanobacteria. During this project period, we will target the production of hydrogen, alkanes and higher alcohols in selected cyanobacterial strains. Specifically, we will use transcriptomics, metabolite profiling, mutagenesis, physiological analysis and genome scale metabolic modeling, all of which will be encased in a systems biology framework.

Thrust 3 — Chemical Catalyst Design

Catalytic Reactivity at the Metal-Solution Interface

Sponsor:	National Science Foundation
Project Leader:	Robert J. Davis, University of Virginia

Supported metal catalysts composed of metal nanoparticles on oxide carriers have been optimized for the upgrading of fossil fuels as well as the production of petrochemicals. However, a recent transition of some chemical processes to utilize biorenewable feedstocks requires new catalysts to be active in liquid water, a reaction medium that is not commonly found in the fossil fuel based industries. Water is a very low cost, highly-polar, environmentally-benign solvent that has great potential as a reaction medium for catalytic transformations of biomass. Indeed, many molecules derived from biomass are highly-oxygenated and quite polar compared to hydrocarbons and are likely to decompose prior to volatilization. Therefore, aqueous-phase catalytic processing of carbohydrate-derived feedstocks is an area of rapidly growing interest in the chemical community. The main objectives of the proposed work are to explore the nature of the catalytic active site and probe the reactivity of adsorbed intermediates on two metal catalyst systems that are highly relevant to the catalytic transformation of biorenewable molecules in liquid water.

GOALI: Understanding Self-Assembly of Nobel Metal Alloys for Ultra Low Temperature Oxidation Catalysis

Sponsor:	National Science Foundation
Project Leader:	Abhaya Datye, University of New Mexico

The treatment of CO and hydrocarbons in the exhaust from lean burn engines is an important challenge facing the wide-spread deployment of high efficiency lean-burn automobiles. Given the increased interest in the use of bio-derived fuels, the development of low-temperature oxidation catalysts supports our nation's efforts to diversify its energy supply and reduce its dependence on foreign fossil fuels. The supplies of precious metals are limited worldwide, but there is increasing demand for clean energy. Hence, there is a need to develop more active catalysts that provide stable performance over long terms at elevated temperatures with minimal use of precious metals. In the present study, we focus on a catalyst system that preserves its composition after elevated temperature treatments, and also preserves catalytic activity over long-term operation. Understanding the principles that lead to this improved behavior could have major impact on the design of industrial catalysts which are involved at some stage in the manufacture of 90% of the products we use today.

Green Catalysis

Sponsor:	National Science Foundation
Project Leader:	L. Keith Woo, Iowa State University

This project is directed towards developing a green chemistry approach to catalytic processes and sustainable, green technologies. To develop more economical and greener catalysts, environmentally friendly transition metal complexes will be explored as substitutes for the more commonly used precious and heavy metal compounds such as ruthenium and rhodium catalysts. A natural source of inspiration and insight into this issue is the world of biology. This will be used in two approaches. The first of these is simply based on the central role of iron porphyrins as the catalytic site in a variety of enzymes. This includes the cytochromes P450 and peroxidases, heme proteins involved in the catalytic transformation of a range of substrates. The wide range of heme use in nature suggested that the utility of synthetic iron porphyrin complexes in catalytic reactions is largely untapped and unappreciated.

Materials for Energy Conversion

Sponsor:	U.S. Department of Energy
Project Leader:	Plamen Atanassov, University of New Mexico
Other Faculty:	Abhaya K. Datye, University of New Mexico

The objective of this collaborative research project is to bridge bio-derived fuels with fuel cell technology as a means of electrical power generation. Biologically-derived fuels promise to be one of the most immediate implementation pathways to relieve the dependence on oil and oil imports. Fuel cells are among the core strategic technologies for energy conversion and electric power generation. There are two ways to link bio-derived fuels with fuel cell technology. One path is through development of fuel reforming and as a source of hydrogen in a way similar to the processes currently used for hydrogen production in petroleum plants. The challenges here are predominantly associated with the complex character of the bio-derived fuels, their chemical composition and the concentration of the biofuel in the feedstock. Thus, effective and selective catalyst development should be accompanied with basic research in reforming reactor engineering in order to both explore the feasibility and prepare the background for the future scale-up and scale-down efforts. Alternatively, ethanol can be used directly as a fuel in DEFC. In this case the catalysis of ethanol oxidation presents the major challenge. Oxidative breaking of the C-C bond should be catalyzed selectively to avoid acetic acid formation, further oxidation of which presents a major obstacle. Catalytic solutions for selective oxidation steps in reforming and selective electrocatalysis of ethanol oxidation are based on the same core chemical phenomena. Therefore, we propose an integrated research program devoted to catalytic reforming and electrocatalyst development. Materials science and technology play a key role in these fuel conversion processes and lessons learned from one of them can be used as guidance for rational catalyst design in the other.

National Advanced Biofuels Consortium (National Renewable Energy Lab Prime)

Sponsor:	U.S. Department of Energy
Project Leader:	Brent Shanks, Iowa State University
Other Faculty:	James Dumesic, University of Wisconsin – Madison

The project involves developing fundamental knowledge on the pyrolysis and catalytic upgrading of biomass to intermediate chemicals that can be integrated into the existing refinery infrastructure. The upgrading portion of the work is focused on catalytic conversion of carbohydrate-derived molecules in the condensed phase.

PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels, Chemicals and Materials

Sponsor:	National Science Foundation
Project Leader:	Abhaya Datye, University of New Mexico
Other Faculty:	Brent Shanks and George Kraus, Iowa State University; James Dumesic, University of Wisconsin – Madison; Robert Davis and Matthew Neurock, University of Virginia; Ib Chorkendorff, Technical University of Denmark; Hans Niemantsverdriet, Eindhoven University of Technology; Dmitry Murzin, Abo Akademi University; and Robert Schlögl, Max Planck Institute

Since this project is of particular importance to achieving the vision of the center and is integral to its strategic research and education plans, a project summary (rather than an abstract) is presented in an earlier section of Volume II.

Selective Hydrogenation of Oxygenates

Sponsor:	Engineering and Physical Sciences Research Council (United Kingdom)
Project Leader:	Robbie Burch and Chris Hardacre (Queens University, Belfast)
Other Faculty:	Matthew Neurock, University of Virginia

This work is focused on understanding the elementary steps that control the hydrogenation of saturated and unsaturated ketones and aldehydes that arise in the processing of biomass intermediates. Theory and simulation are used to understand the influence of solvent, metal, support and the molecule structure on catalytic activity and selectivity.

Structure and Function of Supported Base Catalysts

Sponsor:	U.S. Department of Energy
Project Leader:	Robert J. Davis, University of Virginia

Solid base catalysts exhibit high activities and selectivities for many kinds of reactions important for fuels and chemicals production, including transesterifications, condensations, alkylations, cyclizations, and isomerizations; however, many of these processes are carried out industrially using liquid bases as catalysts. These applications can require nearly stoichiometric amounts of the liquid base for conversion to the desired product. Replacement of liquid bases with solid base catalysts allows for easier separation from the product as well as possible regeneration and reuse. Basic solids also have the added advantages of being non-corrosive and environmentally friendly, which allows for easier disposal. The search for novel solid bases that catalyze transformations with high product selectivity, high reaction rate, and low deactivation rate is an ongoing process. Our research over the past funding cycle involved the exploration of ethanol coupling reactions on solid bases.

The Science and Engineering of Durable Ultra-Low Platinum Group Metal Catalysts

Sponsor:	Los Alamos National Labs (U.S. Department of Energy)
Project Leader:	Abhaya K. Datye, University of New Mexico

The cost and durability of PGM cathode materials is a major barrier to the commercialization of these systems for stationary and transportation power applications. New Ultralow loading PGM cathode materials will be engineered using insights from fundamental science and tested to meet the DOE requirements for mass activity and durability. The UNM portion of this project is focused on appropriate catalyst architectures to maximize the performance of these novel catalysts. Catalyst support interactions and their effects on the durability and mass activity will be investigated.

Research Support – Life Cycle Assessment (LCA)

A Regional Program for Production of Multiple Agricultural Feedstocks and Processing to Biofuels and Biobased Chemicals

Sponsor:	U.S. Department of Agriculture
Project Leader:	Robert Anex, University of Wisconsin – Madison

This project involves a team of university and industry partners led by the LSU AgCenter studying the production of biomass for economically viable conversion to biofuels and bioenergy using existing refinery infrastructure. Through new and existing industrial partnerships, this project will use energy cane and sweet sorghum to help reinvigorate the Louisiana sugar and chemical industries.

Pre-College Education

Iowa EPSCoR: Harnessing Energy Flows in the Biosphere to Build Sustainable Energy Systems

Sponsor:	National Science Foundation
Project Leader:	Theodore J. Heindel, Iowa State University
Other Faculty:	Adah Leshem, Iowa State University

This Research Infrastructure Improvement project seeks to expand the research capacity within the state to support a transition in energy supply from subsurface fossil energy stores to renewable energy flows at or near the earth's surface. The research program is organized into four platforms in renewable energy, each of which consists of several research foci (or planks) which are detailed below. The project seeks to examine energy flows and processes from a holistic systems perspective that considers technical, economic, social, and environmental constraints and impacts. This comprehensive program will enable innovative research, strengthen energy-related education, train a "clean-tech" workforce, and engage diverse communities in implementing environmentally and economically sustainable solutions to the growing energy challenges facing Iowa and the U.S. The project brings together research universities, 2- and 4-year colleges, the private sector, and local government agencies (Iowa Power Fund, Iowa Office of Energy Independence), to address key issues related to renewable energy research, education, and workforce development in the state.

Intellectual Merit: The project seeks to significantly enhance the research competitiveness of Iowa through a comprehensive, multi-faceted research program in renewable energy and energy efficiency. The program builds upon existing strengths of Iowa in the areas of bioenergy and wind energy while including highly complementary components in the areas of energy efficiency and energy policy. The program seeks to change the energy landscape across Iowa by improving the energy balance while mitigating environmental concerns and positioning citizens to compete more effectively in a global economy.

Broader Impacts: An overarching goal of this project is to translate the knowledge gained in the research platforms into specific actions that can increase the participation of under-represented minorities in STEM fields. IA EPSCoR includes the creation of the Future Leaders in Advancing Renewable Energy (FLARE) Institute to lead the implementation of strategic broader impacts activities. Modeled after the Iowa's NSF I3 program, Strengthening the Professoriate at ISU, the FLARE Institute will be a state-wide organization supporting the human infrastructure development needed to accelerate Iowa's transition to a green economy. Critical to this goal are strategies to broaden the participation of women, underrepresented minorities, and first-generation college students in the STEM fields, prepare a workforce that can meet the demands of Iowa's emerging green economy, and create a community of scholars who integrate broader impacts into their research efforts in all fields. Accordingly, the FLARE Institute will address key deficiencies in state-wide infrastructure by integrating broader impacts through all elements of activities leveraged by IA EPSCoR, thereby having a far greater state-wide impact than any individual program could achieve on its own.

In partnership with CBiRC's Summer Academy, the FLARE Institute offers a guided inquiry experience for secondary science school teachers, which provides the tools, experiences, and collaborative relationships necessary for translating the latest developments in STEM (science, technology, engineering, mathematics) into the classroom. During the summer experience, teachers learn laboratory techniques and basic biorenewables concepts through guided experimentation with CBiRC faculty and staff. The program helps middle school teachers discover the nature of science in a research laboratory, and gives teachers a platform for building hands-on learning experiences in their science classrooms. Teachers take real-world knowledge back to their students, and can better relate scientific practices to issues in current events. In 2012, teachers found the Summer Academy program very useful. All teachers agreed that the overall quality of the program was high, that there was positive interaction with other teachers, that there was a positive relationship between the teachers and the instructor, that the program increased teacher content knowledge and understanding of renewable energy, that participation in the program improved teacher confidence to teach students about renewable energy, that the quality of the curriculum resources was high, and that teachers will use information about renewable energy from this program in their classrooms.

Meta!Blast: An Immersive Interactive Learning Module for Cell Biology

Sponsor:	National Institutes of Health
Project Leader:	Eve S. Wurtele, Iowa State University
Other Faculty:	Adah Leshem, Iowa State University

This award-winning videogame teaches about metabolic and energetic biology in the context of a cell. The project website, metablast.org, provides students and teachers with access to the game and various instructional materials. Level 1 is the basic cell and its metabolism; level 2 is the light reactions of photosynthesis. The project includes evaluation in 20 Iowa and Mississippi high schools.

Meta!Blast Awards

- 2012 - Featured at American Association for Advancement of Science Annual Meeting (http://www.aaas.org/meetings/2012/).
- 2012 - International Science and Engineering Challenge: Winner, Honorable Mention, Interactive Videogame. Science Magazine and National Science Foundation
- 2012 - International Science and Engineering Challenge Finalist, Illustration (The Cytosol). Science Magazine and National Science Foundation
- 2010 - Finalist Learning Lab (top 5% of >1,000 submissions), MacArthur Foundation
- 2010 - Second Place, Chlorofilms.

Plants in Society

Sponsor:	National Science Foundation
Project Leader:	Jonathan Wendel, Iowa State University
Other Faculty:	Adah Leshem, Iowa State University

National assessment and evaluation results suggest that K-12 science, technology, engineering and mathematics (STEM) education in our country is in crisis (National Research Council (NRC) 2005, 2009; NAEP, 2009). Despite the importance of formative education, the implementation of high quality elementary school science curricula continues to be a challenge, and elementary teachers struggle with misconceptions about what science is and how to teach it. To address this issue, the Iowa State University Plant Genomics Outreach program has created a socially relevant science workshop, Plants in Society, designed to provide elementary school teachers with the motivation, confidence and resources for inquiry-based curriculum and instruction development. The current study shows elementary school teachers scored significantly better on a content knowledge test covering plant and biorenewables topics after participating in the five-day workshop. A follow-up assessment revealed that, compared to other professional development experiences, teachers felt more inspired to integrate new content and pedagogical techniques into their curricula when basic principles were conveyed to them as community-centered ideas.

University (Graduate and Undergraduate) Education

IINspire LSAMP - An Alliance Modeling How to Broaden Participation in Changing Midwest Demographics

Sponsor:	National Science Foundation
Project Leader:	David K. Holger, Iowa State University
Other Faculty:	Krishna Athreya, Iowa State University

The Iowa (Illinois) Nebraska STEM Partnership for Innovation in Research and Education (IINspire) LSAMP project, under the leadership of Iowa State University, addresses the critical need to broaden the participation of historically underrepresented minorities (URMs) in STEM education throughout Iowa, Nebraska, and a segment of Illinois. This is a new NSF Louis Stokes Alliances for Minority Participation alliance consisting of 16 higher education institutions, nine in Iowa, three in Nebraska, and one in Illinois. The alliance consists of six 2-year institutions, seven private bachelor granting institutions, and three public universities. In addition, there are state agencies, national laboratories, education programs, and companies who are affiliated with the alliance. The IINspire LSAMP has a goal to more than double the number of STEM baccalaureate degrees awarded to URM STEM students within five years and build the foundation for larger increases. Because students fromm populations historically underrepresented in STEM disciplines are the only growing segment of their population, there is a particular statewide urgency to remove the barriers to progress in developing a diverse STEM workforce. This comprehensive and holistic work plan is built on a foundation of available research and resources, focusing on the transitions from high school and community college to

ensure that each student has rigorous academic preparation, social support, research preparation, and financial support to complete their STEM degree and continue onto graduate school. The current increase in the number of African-Americans, Hispanics, Native Americans and Native Hawaiian/Pacific Islanders entering freshman and community college transfers into STEM fields provides evidence that Iowa has begun to address all aspects of student success from the final years of high school through entrance into undergraduate studies. The proposed LSMAP activities will further understand these transitions, develop additional models to increase the number of students at these entrance points and nurture them through the completion of the STEM degree with a set of structures and monitored activities. Critical new programs include a research certification, community based recruiting and attention to STEM pedagogy. The alliance is designed to connect these new activities to existing programs within the participating institutions in order to leverage the resources and expertise. Finally, the program and student progress will be evaluated for continuous improvement. The partnership with the Science Education Resource Center (SERC) at Carleton College in Minnesota will provide quick access to dissemination of successful practices in STEM recruitment and retention. The overall project plan will result in contributions to further research supported models to broaden participation regionally in Midwestern states and nationally overall.

UCI Biomedical Informatics Training Undergraduate Summer Research (BIT-SR) Program

Sponsor:	National Institutes of Health
Project Leader:	Pierre Baldi, University of California – Irvine
Other Faculty:	Suzanne Sandmeyer, University of California – Irvine

The UCI Biomedical Informatics Training Undergraduate Summer Research (BIT-SR) Program for undergraduates will be coordinated with and supported by the infrastructure of the UCI Biomedical Informatics Training (BIT) Program, a campus-wide NIH/NLM-supported training program administered by the UCI Institute for Genomics and Bioinformatics. The BIT Program provides in-depth training to graduate students and postdoctoral fellows in either computational or life sciences, and trains them to working competence in the cross-discipline with an emphasis on cutting-edge research in biomedical informatics and computational biology approaches to problems in areas such as molecular biology and biochemistry, pharmacology and drug discovery, genomics, proteomics, structural biology, and systems biology, particular areas of strength at UCI. It is the goal of the BIT program to prepare world class computational and life scientists in their primary fields with sufficient cross- training for interdisciplinary collaboration at a scholarly level. Each student in the BIT program is mentored by two faculty members, one from the computational sciences and one from the life sciences: a thesis advisor from the student's home Department, and a co-advisor in the cross-discipline with ongoing collaborations with the student's thesis advisor in areas related to the student's thesis research. Each BIT student performs his/her thesis research in collaboration with a graduate student in the cross-discipline in the co-advisors lab. Based on their research interests, each BIT-SR Program student will be assigned to a faculty-advisor in his/her area of interest and teamed with a BIT graduate student in that advisors laboratory who will serve as the student's immediate research mentor and collaborator. Throughout their summer research experience, each BIT-SR Program student will participate in all individual laboratory activities such as laboratory meeting presentations,

departmental seminar attendance, etc. At the end of the summer research period, each student will prepare a written paper of their work and present their results at a BIT Undergraduate Summer Research Program Symposium attended by UCI/IGB faculty and students and the UCI research community at large. The University of California at Irvine (UCI) enjoys a national reputation both for the excellence of its undergraduate research programs and for its NIH/NLM-supported Biomedical Informatics Training (BIT) Program for graduate students and postdoctoral fellows. Here we propose to unite and extend these well-established and highly successful programs to model a high quality summer research experience for community college students from low-income, first generation, and historically underserved ethnic and racial backgrounds from two-year colleges in the greater Los Angeles area, to encourage these students to pursue research training and career opportunities in rapidly emerging fields at the interface of the computational and life sciences.

In the summer of 2012, two students worked in the Sandmeyer laboratory: Monica Singha on designing vectors for use in *Yarrowia*, and Isaac Batty on development of an automatic search engine for assembling enzyme kinetic data for use in metabolic modeling.

Data Management Plan

NSF Engineering Research Center for Biorenewable Chemicals (CBiRC)

Pursuant to NSF Cooperative Agreement Financial & Administrative Terms and Conditions (FATC) Article 40, *Sharing of Findings, Data, and Other Research Products*, CBiRC strives to support the prompt publication of significant findings from research and education activities. CBiRC also strives to ensure that authorship accurately reflects the contributions of those involved. CBiRC encourages its investigators to share with other researchers, at no more than incremental cost and within a reasonable time, the data, samples, physical collections and other supporting materials created or gathered in the course of the work. CBiRC also encourages awardees to share software and inventions or otherwise act to make the innovations they embody widely useful and usable.

CBiRC recognizes that adjustments and, where essential, exceptions may be allowed to safeguard the rights of individuals and subjects, the validity of results, or the integrity of collections or to accommodate legitimate interests of investigators.

The following plan articulates how findings, data and other research materials that have resulted fully or in part from activities supported by the NSF Engineering Directorate to CBiRC under Award No. EEC-0813570 — or by extension, through mandatory cost sharing and membership fees/revenues generated as a result of the Center's industry program — will be implemented. It outlines the rights and obligations of all parties as to their roles and responsibilities in the management and retention of said data.

Expected Data

All faculty and staff at each institution have responsibility for identifying and retaining university records — paper and electronic — in accordance with the Records Retention Guidance and Schedule. Consistent with Iowa Code §305.2(9), Iowa State University records are defined as any document, book, paper, electronic record, photograph, sound recording, or other material, regardless of physical form or characteristics, containing information, and which is made, produced, executed, or received in connection with the transactions and activities of the university. All faculty and staff have responsibility for complying with the provisions of the Records Retention Schedule which addresses the management and preservation of specific university record types. The Schedule indicates the required:

- Duration for which each record type must be retained
- Responsibility assignments for the management of active records, the storage of inactive records, and the archival of permanent records
- Confidentiality of each record type
- Disposal method (if applicable)
- Contact information for submitting additions, updates, and corrections to the Retention Schedule

The types of primary data, samples, collections, software, curriculum, and other materials that are produced in the course of the Center's research are listed below. Primary research data are defined as "recorded factual material commonly accepted in the scientific community as necessary to validate research findings." Hence, the basic level of digital data to be archived and

made available includes (1) analyzed data and (2) the metadata that define how these data are generated. These are data that are, or should be, published in theses, dissertations, refereed journal articles, supplemental data attachments for manuscripts, books and book chapters, and other print or electronic publication formats.

1. Analyzed data are (but are not restricted to) digital information that would be published, including digital images, published tables, and tables of the numbers used for making published graphs.
2. Necessary metadata are (but are not restricted to) descriptions or suitable citations of experiments, apparatuses, raw materials, computational codes, and computer-calculation input conditions.

The Office of Management and Budget statement (1999) specifies that the definition above does not include preliminary analyses, drafts of scientific papers, plans for future research, peer reviews, and communications among colleagues. Raw data fall into this category as preliminary analyses. These types of data are all therefore excluded, as are proprietary or other restricted data and data derived from human subjects research, since under human subject protocols, there is a requirement to protect privacy and confidentiality.

Period of Data Retention

The data described above will be managed and retained for a period of three years beyond the end date of the NSF award. Hence, the effective period is 9/1/2008 to 8/31/2019.

Data Formats and Dissemination

Dissemination approaches that will be used to make data available to others include CBiRC annual reports, CBiRC publications, and deposition of theses in university libraries according to standard practices and policies at each institution.

- Policies for public access and sharing include standard practices and policies in place at each institution.
- CBiRC recognizes that in some instances, such as those involving development of commercially applicable or patentable products or techniques, disclosure of results from certain research projects may need to be withheld for a limited period of time.
- Sharing and management of data with center members, institutional partners, and other major stakeholders will involve regular center-wide presentations, discussions, publications, reports and annual reports and will be made available in an internal members-only SharePoint site (CBiRC intranet).

Data Storage and Preservation of Access

The physical and cyber resources and facilities that will be used for the effective preservation and storage of research data, including that derived from the Center's subcontractors, are administered by the Records Retention Policy Administrator. The University Records Retention Schedule exists to provide the university community with guidance on the retention and disposal of university records. The schedule supports the university's Records Retention Policy and establishes retention periods based on the content and purpose of university records. The University Records Retention Schedule:

- Describes various types of records existing within the University.

- Specifies the duration that records of a given type are to be retained.
- Applies to records identified as "university records" based on their purpose and content.
- Applies to university records without regard to the form or media in which the records exist (e.g., paper, email, server, tape, disk).
- Indicates the department or other unit responsible for the management and storage of retained records.
- Indicates for each record type a "data classification" which guides security practices for active and archived records, and the disposal of expired records. Center members are referred to the Records Retention Policy for cautions related to destruction of university records.

Post-Award Monitoring

This data management plan will be monitored primarily through the normal annual and final report process and through evaluation of subsequent proposals.

Expected Data	Period of Data Retention	Data Formats and Dissemination	Data Storage and Preservation of Access	Post-Award Monitoring
Copyrightable material, including: • Reports • Books • Journal articles • Software • Databases • Sound- and video-recordings	Three years after termination of the NSF award. The effective period of award is 9/1/2008 to 8/31/2016, meaning that the period of data retention will extend to 8/31/2019.	As lead institution, ISU will be responsible for assuring that the cognizant NSF Program Officer is provided access to, either electronically or in paper form, a copy of every publication of material based on or developed under this award, clearly labeled with the award number and other appropriate identifying information, promptly after publication.	Electronic archives of data generated will be maintained using standard university policies and practices in place at Iowa State University as well as the other institutions associated with CBiRC.	Post-award monitoring will be based upon standard university policies and practices in place at Iowa State University as well as the other institutions associated with CBiRC.
Technology transfer products, including: • Invention disclosures • Patent applications • Patents awarded • Licenses issued • Materials subject to Material Transfer Agreements	Depending on the technology transfer product involved, three to 10 years after termination of the NSF grant. Thus, the termination date will be either 8/31/2019 or 8/31/2026.	Any invention disclosures or patents or other technology transfer products will be formatted and managed using standard university practices as dictated by the various Technology Transfer offices.	Electronic archives of invention disclosures or patents or other technology transfer products will be maintained diligently and professionally using university and company servers and back-up hard drives.	Post-award monitoring will be based upon standard university policies and practices in place at Iowa State University as well as the other institutions associated with CBiRC.

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(a) Professional Preparation

University of California, Davis	Mechanical Engineering	B.S., 1981
University of California, Davis	Mechanical Engineering	M.S., 1983
University of California, Davis	Environmental Engineering	Ph.D., 1995

(b) Appointments

2010 – present	Professor, Biological Systems Engineering, University of Wisconsin, Madison
2009 – 2010	Professor, Agricultural & Biosystems Engineering, and Mechanical Engineering, Iowa State University
2003 – 2009	Assoc. Professor, Agricultural & Biosystems Engineering, and Mechanical Engineering, Iowa State University
2005 – 2009	Assoc. Director, Bioeconomy Institute, Iowa State University
2002 – 2003	Assoc. Professor, Aerospace & Mechanical Engineering, and Research Fellow, Institute for Science and Public Policy, University of Oklahoma
1996 – 2002	Asst. Professor, Aerospace & Mechanical Engineering, and Research Fellow, Institute for Science and Public Policy, University of Oklahoma
1989 – 1991	Senior Engineer & Section Head, Systems Control Technology, Inc., Palo Alto, CA
1983 – 1989	Research Engineer, Systems Control Technology, Inc., Palo Alto, CA

(c) Products

i. Five products most closely related to the proposed project

1. Raman, D. R., R. P. Anex. 2012. Conceptual and mathematical models of batch simultaneous saccharification and fermentation: dimensionless groups for predicting process dynamics. *Journal of Biological Systems* 20(2): 195 – 211.
2. Kabir F. K., A. D. Patel, J. C. Serrano-Ruiz, J. A. Dumesic, R. P. Anex. 2011. Techno-economic analysis of Dimethylfuran (DMF) and Hydroxymethylfurfural (HMF) production from pure fructose in catalytic processes. *Chemical Engineering* 169(1-3): 329-38
3. Patel, A. D., J. C. Serrano-Ruiz, J. A. Dumesic, R. P. Anex. 2010. Techno-economic analysis of 5-nonanone production from levulinic acid. *Chemical Engineering Journal* 160: 311-321.
4. Anex, R. P., A. Aden, F. K. Kazi, J. Fortman, R. M. Swanson, M. M. Wright, J. A. Satrio, R. C. Brown, D. E. Daugaard, A. Platon, G. Kothandaraman, D. D. Hsu, A. Dutta. 2010. Techno-economic comparison of biomass-to-transportation fuels via pyrolysis, gasification, and biochemical pathways. *FUEL* 89 (2010): S29-S35. doi:10.1016/j.fuel.2010.07.015
5. F. K. Kazi, J. Fortman, R.P. Anex, D.D. Hsu, A. Aden, A. Dutta, G. Kothandaraman. 2010. Techno-economic comparison of process technologies for biochemical ethanol production from corn stover. *FUEL* 89 (2010): S20–S28. doi:10.1016/j.fuel.2010.01.001.

ii. Five other significant products

1. Anderson, C. J., R. P. Anex, R. W. Arritt, B. K. Gelder, S. Khanal, D. E. Herzmann, P. W. Gassman. 2013. Regional climate impacts of a biofuels policy projection. *Geophysical Research Letters* (DOI: 10.1002/grl.50179).
2. Bennett, A.S. and R.P. Anex. 2009. Production, Transportation and Milling Costs of Sweet Sorghum as a Feedstock for Bioethanol Production. *Bioresource Technology*, Volume 100(4):1595-1607.
3. Heggenstaller, A.H., R.P. Anex, M. Liebman, D.N. Sundberg, L.R. Gibson. 2008. Productivity and nutrient dynamics in bioenergy double-cropping systems. *Agronomy Journal* 100(6):1740-1748.
4. Isci, A., J. N. Himmelsbach, J. Strohl, A. L. Pometto III, D. R. Raman, R. P. Anex. 2008. Pilot scale fermentation of aqueous ammonia soaked switchgrass. *Applied Biochemistry and Biotechnology* 144: 69-77.
5. Anex, R.P., L.R. Lynd, M.S. Laser, A.H. Heggenstaller, M. Liebman. 2007. Potential for enhanced nutrient cycling through the coupling of agricultural and bioenergy systems. *Crop Science Journal* 47:1327-1335.

(d) Synergistic Activities

Life Cycle Assessment Lead, USDA/AFRI Bioenergy CAP project, 2011-2016
Systems Analysis Lead, USDA/AFRI Corn Systems CAP project, 2011-2016
Member, NRC Committee on Sustainability Linkages in the Federal Government, 2011-2013
Member, Science and Technology for Sustainability Subcommittee of U.S. EPA Board of Scientific Counselors, providing expertise on life-cycle analysis of biorenewable systems, 2009-2010
Associate Editor, *Intl. Journal of Life Cycle Assessment* and *Journal of Industrial Ecology*.

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Aden, A.	Natl. Renewable Energy Lab	Liebman, M.	Iowa State University
Birrel, S.	Iowa State University	Lynd, L.	Dartmouth College
Coors, J.	University of Wisconsin	Moore, K.	Iowa State University
Dumesic, J.	University of Wisconsin	Muck, R.	USDA/ARS
Hatfield, J.	USDA/NSTL	Raman, D.R.	Iowa State University
Hess, J.R.	DOE/INL	Richard, T.	University of Pennsylvania
Hinrichs, C.	University of Pennsylvania	Sheehan, J.	University of Minnesota
Hsu, D.	Natl. Renewable Engergy Lab	Shinners, K.	University of Wisconsin

ii. Graduate Advisors and Post-Doctoral Sponsors

Englehardt, J. University of Miami Hubbard, M. UC-Davis Lund, J. UC-Davis

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

Students

A. Isci (Ph.D. 2008, Mascoma), K. Edwards (M.S. 2008), A. Heggenstaller (Ph.D. 2009, MRI), J. Fortman (M.S. 2009), A. Patel (M.S. 2009), Y-N Wu (M.S. 2009), S. Khanal (Ph.D. 2012)

Current Students

S. Dhungel, Ph.D., S. Gunukula, Ph.D., E. Oritz-Reyes, Ph.D., L. Gu, M.S., A. Li, M.S., T. Gunawardhana, M.S.

Postdoctoral Associates

Dr. F. Kabir Kazi (2008-10), Dr. B. Gelder (2008-10), Dr. Rashid Rafique (2011-13), Dr. M. Necpalova (current)

28 = Total number of graduate students advised and postdocs sponsored

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(a) Professional Preparation

Cotton College, Guwahati, Assam, India	Physics	B.Sc. & M. Sc., 1976
Iowa State University	Condensed Matter Physics	Ph. D. , 1986
Iowa State University	Superconductivity	1987-1989

(b) Appointments

May 2011-April 2012	Diversity Co-Director, CBiRC
March 2006-May 2010	Director, Engineering Leadership Program Adjunct Associate Professor, Materials Science and Engineering Iowa State University
Feb 2000-June 2004	Director, Women's Programs in Engineering, Cornell University
May 2001-Aug 2003	Interim director, Minority Programs in Engineering, Cornell University
March 1993-Feb 2000	Coordinator, Program for Women in Science and Engineering, Iowa State University
Aug 1987-May 1992	Temporary Assistant Professor, Department of Physics, Iowa State University
June 1988-May 1989	Post-doctoral Research Associate, Ames Laboratory, DOE, Iowa State University

(c) Products

i. Five products most closely related to the proposed project

1. Krishna S. Athreya, Nidhi Bhandari, Michael T. Kalkhoff, Diane T. Rover, Alexandra M. Black, Elif Eda Miskioğlu, and Steven K. Mickelson, *Work in Progress:Engineering Leadership Program: A Thematic Learning Community*, Proc. 40th ASEE/IEEE Frontiers in Education Conference, F2D-1, 2010
2. Elif Eda Miskioğlu, Krishna S. Athreya, Nidhi Bhandari, Michael T. Kalkhoff, Diane T. Rover, Alexandra M. Black, Nathan D. Meisgeier, *Engineering Leadership Program: The First Year Experience*, Proc. 40th ASEE/IEEE Frontiers in Education Conf., T4E-1, 2010
3. Michael Kalkhoff, Krishna S. Athreya, Diane Rover, Steven K Mickelson and Amy Joines, "*Highlights and Challenges of a Student Driven Co-Curricular Leadership Program*, Proc. 39th ASEE/IEEE Frontiers in Education Conf., session T4F-1, 2009
4. Krishna S. Athreya, Michael Kalkhoff, Gregory McGrath, Adam Bragg, Amy Joines, Diane T. Rover and Steven Mickelson, *Work in Progress: Engineering Leadership Program: Tracking LeadershipDevelopment of Students using Personalized Portfolios*, Proc. 38th ASEE/IEEE Frontiers in Education Conference, F3C-11, 2008.
5. Krishna S. Athreya, Diane Rover, Sarah Walter, Steven K. Mickelson, Gregory McGrath, Michael Kalkoff, Tyler Rasmussen, Gloria Starns, Rhonda Wiley-Jones, Kevin Saunders, Mack Shelley, *Work in Progress-Progression of an Engineering Leadership Program for the Future*, Proc. 37th ASEE/IEEE Frontiers in Education Conference, T2J-16, 2007.

ii. Five other significant products

1. Ryan Legg, Mark Tekippe, Krishna S. Athreya, and Mani Mina, *Solving Multidimensional Problems Through a new Perspective: The integration of design for sustainability and engineering education,* Proc. ASEE Annual Conference, 2005
2. Yalem Teshome, Nancy Maushak and Krishna Athreya, *Attitude Toward Informal Science and Math: A Survey of boys and Girls participating in hands-on science and Math (Funtivities)*, Journal of Women and Minorities in Science and Engineering, **7,** 59 (2001).
3. Kit-Yee Daisy Fan, K. S. Athreya and R. J. Burt, *The CURIE River Basin: Introduction to Engineering in a Social Context,* Proceedings of the ASEE Annual Conference, 2001.
4. K. S. Athreya, S. C. Sanders, D. Hofreiter, and D. K. Finnemore, "Critical-Current-Free-Energy Relations in High T_C Superconductors," Phys. Rev. B **40**, 264(1990).
5. K. S. Lichtenberger, S. C. Sanders, K. S. Athreya, O. B. Hyun, and D. K. Finnemore, "Factors that Control J_C in High T_C Superconductors," Proceedings of Critical Currents in High T_C Superconductors, Karlsruhe, FRG, 1989, Cryogenics **30**, 846(1990).

(d) Synergistic Activities

Reviewer, ED grants for Hispanic Serving Institutions, 2012
Reviewer, Judge, Emerging Researchers National Conference, 2012, 2013
Teaching Responsible Communication of Science, Science Advisory Board, 2012-2013
President (also co-founder) *Engineers for a Sustainable World (formerly, Engineers Without Frontiers)*, www.eswusa.org, 2002-2010
Facilitator, Dialogues in Diversity course, Iowa State University, 2004-2006
Member, Action Planning Committee, Indo US Collaboration for Engineering Education (IUCEE), 2007
Special Advisor, Committee on Opportunities in Science (COOS), American Association for the Advancement of Science (AAAS), 2009-present
Board appointed member of COOS committee, AAAS, 2003-2006 and 2006-2009
Advisory Committee, *Access to Advancement: An Audio Exploration of the National Effort to Increase the Role of Women with Disabilities in Science, Technology, Engineering, and Mathematics (STEM),*WAMC Northeast Public Radio, 2008-2010

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Diane Rover	IINSPIRE LSAMP Director
Derrick Rollins	IINSPIRE LSAMP Director
Alyssa Burger	Center for Compact and Efficient Fluid Power
Ms. Nell Brady	WAMC Northeast Public Radio
Mr. Glenn Busby	WAMC Northeast Public Radio
Dr. Yolanda George	American Association for the Advancement of Science
Dr. Shirley Malcolm	American Association for the Advancement of Science

ii. Graduate Advisors and Postdoctoral Sponsors (your own)

Dr. Robert N. Shelton	Executive Director, Fiesta Bowl Graduate Advisor)
Dr. Douglas Finnemore	Retired Professor Emeritus, Iowa State University

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

N/A

ADAM BARB
Assistant Professor, Biochemistry, Biophysics & Molecular Biology
Iowa State University
2214 Molecular Biology, Ames, IA 50011
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A. Professional Preparation

University of Georgia	Physical Chemistry	PostDoc, 2008-12
Duke University	Biochemistry	Ph.D., 2008
North Carolina State University	Plant Physiology	M.S., 2002
Purdue University	Horticulture Science	B.S., 2000

B. Appointments

2012-	Assistant Professor, Biochemistry, Biophysics & Mol Biol, Iowa State University
2010-12	NIH Ruth L. Kirschstein Research Fellow, University of Georgia
2002-04	Adjunct Instructor, Biology, Meredith College
2002-04	Associate Scientist, Vector Research LLC.

Selected Honors and Awards

2012	NIH Career Development Award (K22)
2012	University of Georgia Outstanding Postdoc Research Presentation
2011	University of Georgia Postdoctoral Research Award
2010	NIH Ruth L. Kirschstein Fellowship (F32)

C. Products (out of a total of 21)

(i) Five products most closely related to the proposed project

1. A. W. Barb, M. Lu, K. W. Moremen, J. H. Prestegard "NMR characterization of Immunoglobulin G Fc glycan motion on enzymatic sialylation" *Biochemistry* (2012).
2. A. W. Barb, D. I. Freedberg, M. D. Battistel, J. H. Prestegard "NMR detection and characterization of sialylated glycoproteins and cell surface polysaccharides*" J Biomol NMR* (2011).
3. A. W. Barb, J. H. Prestegard "NMR analysis demonstrates the immunoglobulin G N-glycans are accessible and dynamic" *Nature Chem Biol* (2011).
4. A. W. Barb, A. J. Borgert, M. Liu, G. Barany, D. Live "Intramolecular glycan-protein interactions in glycoproteins" *Meth Enz* (2010).
5. A. W. Barb, E. K. Brady, J. H. Prestegard "Branch-specific sialylation of IgG-Fc glycans by ST6-Gal-I" *Biochemistry* (2010).

(ii) Five other significant products

1. A. W. Barb, S. K. Hekmatyar, J. N. Glushka, J. H. Prestegard "Exchange facilitated indirect detection of hyperpolarized $^{15}ND_2$-amido-glutamine" *J Mag Res* (2011).
2. A. W. Barb, T. L. Leavy, L. I. Robins, Z. Guan, D. A. Six, P. Zhou, C. R. Bertozzi, C. R. H. Raetz "Uridine-based inhibitors as new leads for antibiotics targeting *E. coli* LpxC" *Biochemistry* (2009).
3. A. W. Barb, L. Jiang, C. R. H. Raetz, and P. Zhou "Structure of the deacetylase LpxC bound to the antibiotic CHIR-090: time-dependent inhibition and specificity in ligand binding" *PNAS* (2007).
4. A. W. Barb, A. L. McClerren, S. Karnam, C. M. Reynolds, P. Zhou, C. R. H. Raetz "Inhibition of lipid A biosynthesis as the primary mechanism of CHIR-090 antibiotic activity in *Escherichia coli*" *Biochemistry* (2007).
5. H. Koiwa, A. W. Barb, L. Xiong, F. Li, M. G. McCully, B. H. Lee, I. Sokolchik, J. Zhu, Z. Gong, M. Reddy, A. Sharkhuu, Y. Manabe, S. Yokoi, J. K. Zhu, R. A. Bressan, P. M. Hasegawa "C-terminal

domain phosphatase-like family members (AtCPLs) differentially regulate *Arabidopsis thaliana* abiotic stress signaling, growth, and development" *PNAS* (2002).

D. Synergistic Activities

- Investigator, Center for Biorenewable Chemicals, Iowa State University

E. Collaborators and Other Affiliations

Collaborators and Co-editors (last 48 months)

Kelly Moremen, Complex Carbohydrate Research Center, University of Georgia; William Lanzilotta, Biochemistry, University of Georgia; Martin Frank, University of Goteborg; Daron Freedberg, FDA/NIH.

Graduate Advisors and Postdoctoral Sponsor

Graduate: D.Mason Pharr and John Williamson (NCSU); Pei Zhou and Christian Raetz (Duke); *Postdoctoral*: James Prestegard (UGA)

Thesis Advisor (1)

Quilin Hanson, ISU BBMB

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Iowa State University
2152 Molecular Biology Bldg., Ames, IA 50011
515-294-4165 / 515-294-0453 (fax) / bobik@iastate.edu

(a) Professional Preparation

Indiana University, Bloomington, IN	Microbiology	B.S., 1979
University of Illinois, Urbana, IL	Microbiology	M.S., 1986
University of Illinois, Urbana, IL	Microbiology	Ph.D., 1990

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(b) Appointments

2009-present	Professor	Biochemistry, Biophysics and Mol. Biol. Iowa State University, Ames, IA
2004-2009	Associate Professor	Biochemistry, Biophysics and Mol. Biol. Iowa State University, Ames, IA
1995-2003	Assistant Professor	Microbiology and Cell Science University of Florida, Gainesville, FL
1990-1995	Postdoctoral Fellow	University of Utah, Salt Lake City, UT Advisor, John R. Roth
1985-1990	Graduate Student	University of Illinois, Urbana, IL Advisor, Ralph S. Wolfe

(c) Products (58 total)

i. Five products most closely related to the proposed project

1. Zhu H, Gonzalez R, and **Bobik T. A.** (2011) Co-production of acetaldehyde and hydrogen during glucose fermentation by *Escherichia coli*. Appl. Environ. Microbiol. 77, 6441-6450.
2. Gogerty, D.S. and **Bobik, T.A.** (2010) Formation of isobutene from 3-hydroxy-3-methylbutyrate by diphosphomevalonate decarboxylase. Appl. Environ. Microbiol. 76, 8004-8010
3. Fan C, Cheng S, Sinha S, **Bobik T.A**. (2012) Interactions between the termini of lumen enzymes and shell proteins mediate enzyme encapsulation into bacterial microcompartments. PNAS 109, 14995-5000
4. Cheng S, Fan C, Sinha S, **Bobik T.A**. (2012) The PduQ enzyme is an alcohol dehydrogenase used to recycle NAD+ internally within the Pdu microcompartment of *Salmonella enterica*. PLoS One. 7, e47144
5. Fan, C., Cheng, S., Liu, Y. , Escobar, C. M., Crowley,C. S., Jefferson, R. E., Yeates, T. O., and **Bobik, T. A.** (2010) Short N-terminal sequences target proteins into bacterial microcompartments. PNAS 107,7509-7514.

ii. Five other significant products

1. Liu, Y., Leal, N. A., Sampson, E. M., Johnson, C. L. V., Havemann, G. D. and **Bobik, T. A.** (2007) PduL is an evolutionarily distinct phosphotransacylase involved in B_{12}-dependent 1,2-propanediol degradation by *Salmonella enterica* serovar Typhimurium LT2. J. Bacteriol. 185,1589-1596.
2. Leal, N. A., Olteanu, H., Banerjee, R., and **Bobik, T. A**. (2004) Human ATP:Cob(I)alamin adenosyltransferase and its interaction with methionine synthase reductase. J. Biol. Chem. 279, 47536-47542.

3. Leal, N. A., Park, S. D., Kima, P. E. and **Bobik, T. A.** (2003). Identification of the human and bovine ATP:Cob(I)alamin adenosyltransferase cDNA based on complementation of a bacterial mutant. J. Biol. Chem. 278, 9227-9234.
4. Havemann, G. D. and **T. A. Bobik**. (2003) Protein content of the polyhedral organelles involved in coenzyme B_{12}-dependent degradation of 1,2-propanediol in *Salmonella enterica* serovar Typhimurium LT2. J. Bacteriol. 185, 5086-5095.
5. **Bobik, T. A**. and Rasche, M. E. (2001) Identification of the human methylmalonyl-CoA racemase gene based on the analysis of prokaryotic gene arrangements: implications for decoding the human genome. J. Biol. Chem. 276, 37194-37198

(d) Synergistic Activities

Dr. Bobik's specialty is bacterial genetics and physiology. He is currently conducting fundamental studies on vitamin B_{12} metabolism and bacterial microcompartments as well as applied studies on bacterial metabolic pathway engineering. The applied studies include genetic engineering of *Escherichia coli* for the production of renewable chemicals, and the construction of novel pathways for production of bioethanol and advanced biofuels.

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Dr. Todd Yeates, UCLA

ii. Graduate Advisors and Postdoctoral Sponsors

Ralph S. Wolfe, Graduate advisor, University of Illinois, Urbana-Champaign
John R. Roth, Post-doctoral advisor, University of California, Davis

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

Zhu, Huilin, PhD student, 2012	WuXi AppTec (pharmaceuticals)
Gogerty, David, PhD student, 2012	Global Bioenergies
Cheng, Shouqiang, PhD, 2011	Northwestern University, Chicago, IL
Fan, Chenguang, PhD, 2010	Yale University
Liu, Yu, MS student, 2009	UC Berkeley
Sinha, Sharmistha, Post-doctoral, current	ISU
Chowdhury, Chiranjit, Post-doctoral, current	ISU
Lehman, Brent, PhD student, current	ISU
Liu, Yu, PhD student, current	ISU
Alexandra Volker, MS student, Current	ISU
Fan, Chenguang, Post-doctoral, 2010-2012	ISU
Gogerty, David, Post-doctoral, 2012	ISU
Cheng, Shouqiang, Post-doctoral, 2011-2012	ISU
Hennen-Bierwagon, Tracie, Post-doctoral, 2009	ISU

14 = Total number of graduate students advised and postdocs sponsored last 5 years.

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(a) Professional Preparation

University of Massachusetts	Chemical Engineering	B.S., 1982
California Institute of Technology	Chemical Engineering	M.S., 1985
California Institute of Technology	Chemical Engineering	Ph.D., 1988

(b) Appointments

2004 – present	Professor, Biomedical Engineering (joint appointment), UC Irvine
2000 – present	Professor, Chemical and Biochemical Engineering, UC Irvine
1994 – 2000	Assoc. Professor, Chemical and Biochemical Engineering, UC Irvine
1988 – 1994	Asst. Professor, Chemical and Biochemical Engineering, UC Irvine

(c) Products

i. Five products most closely related to the proposed project.

1. M.Y.W. Shen, F. Fang, S. Sandmeyer*, N.A. Da Silva*. 2012. Development and characterization of a vector set with regulated promoters for systematic metabolic engineering in *Saccharomyces cerevisiae*. *Yeast*. **29**, 495-503.
2. N.A. Da Silva*, S. Srikrishnan. 2012. Minireview: Introduction and expression of genes for metabolic engineering applications in *Saccharomyces cerevisiae*. *FEMS Yeast Research*. **12**, 197-214.
3. F. Fang, K. Salmon, M.W.Y. Shen, K.A. Aeling, E. Ito, B. Irwin, U. Tran, G.W. Hatfield, N.A. Da Silva*, S. Sandmeyer*. 2010. A vector set for systematic metabolic engineering in *Saccharomyces cerevisiae*. *Yeast*, **28**, 123-136.
4. S.M. Ma, J.W.-H. Li, J.W. Choi, H. Zhou, K.K.M. Lee, V.A. Moorthie, X. Xie, J.T. Kealey, N.A. Da Silva, J.C. Vederas*, Y. Tang*. 2009. Complete reconstitution of a highly reducing iterative polyketide synthase. *Science*, **326**, 589-592.
5. K.M. Lee, N.A. Da Silva*, J.T. Kealey*. 2009. Determination of the extent of phosphopantetheinylation of polyketide synthases expressed in *Escherichia coli* and *Saccharomyces cerevisiae*. *Analytical Biochemistry*, **394**, 75-80.

ii. Five other significant products

1. S. Srikrishnan, W. Chen, N.A. Da Silva*. 2013. Functional characterization and assembly of a modular xylanosome for hemicellulose hydrolysis in yeast. *Biotechnol. Bioeng*. **110**, 275-285.
2. S.-L. Tsai, N.A. Da Silva, W. Chen*. 2013. Functional display of complex cellulosomes on the yeast surface via adaptive assembly. *ACS Synth. Bio*. **2**, 14-21.
3. S. Srikrishnan, A. Randall, P. Baldi, N.A. Da Silva*. 2012. Rationally selected single-site mutants of the *Thermoascus aurantiacus* endoglucanase increase hydrolytic activity on cellulosic substrates. *Biotechnol. Bioeng*. **109**, 1595-1599.
4. S.W.P. Chan, S.-P. Hung, S.K. Raman, G.W. Hatfield, R.H. Lathrop, N.A. Da Silva*, S.-W. Wang*. 2010. Recombinant human collagen and biomimetic variants using a de novo gene optimized for modular assembly. *Biomacromolecules*. **11**, 1460-1469.
5. W. Lee and N.A. Da Silva*. 2006. Application of sequential integration for metabolic engineering of 1,2-propanediol production in yeast. *Metabolic Engineering*, **8**, 58-65.

(d) Synergistic Activities

UCI ADVANCE Program (Sponsored by the NSF ADVANCE Program): Service as Equity Advisor for The Henry Samueli School of Engineering working with the Dean, Department Chairs, Search Committees on the recruitment, retention, and advancement of women faculty. Responsibilities include the development of Assistant Professor mentoring programs, and organizing panels and meetings for women students.

Service on editorial boards: *J. Biotechnol.*, *Appl. Biochem. Biotechnol*., *FEMS Yeast Res*.

BioEMB: Bioengineering Educational Materials Bank, Member of Advisory Board.

Took the lead in outlining and implementing curriculum changes for the undergraduate Chemical Engineering major. Led ABET accreditation activities.

(e) Collaborators & Other Affiliations

i. Collaborators (past 48 months)

Pierre Baldi, Professor, Arlo Randall, Ph.D.; University of California, Irvine
Rachel Chen, Professor; Georgia Tech
Wilfred Chen, Professor; University of California, Riverside
G. Wesley Hatfield, Professor; University of California, Irvine
James E. Kealy, Ph.D., formerly at Kosan Biosciences
Richard H. Lathrop, Professor; University of California, Irvine
Kirsty Salmon, Ph.D.; Verdezyne
Suzanne Sandmeyer, Professor; University of California, Irvine
Hyun Shik Yun, Professor; Inha University
Yi Tang, Professor; University of California, Los Angeles
Sheryl Tsai, Professor; University of California, Irvine
John C. Vederas, Professor; University of Alberta, Canada
Szu-Wen Wang, Assistant Professor; University of California, Irvine

ii. Graduate Advisor

James E. Bailey (deceased)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

Current Postgraduate-Scholar:
Abeer Jabalah

Past Students:
Sam Wei Polly Chan (Ph.D. – 2012), Michael W.Y. Shen (Ph.D. – 2012), Sneha Srikrishnan (Ph.D. – 2012), Manely Kouhssari (M.S. – 2008)

Current Students:
Jin Wook Choi, Christopher Leber, Javier Cardenas, Ruben Fernandez Moya, Richard Que, Kanchana Sridhar

32 = Total Number of Graduate Students Advised and Postdoctoral Associates Sponsored

ABHAYA K. DATYE
Distinguished Regents Professor, Chemical & Nuclear Engineering Department
University of New Mexico
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(a) Professional Preparation

Indian Institute of Technology	Chemical Engineering	B.S., 1975
University of Cincinnati	Chemical Engineering	M.S., 1980
University of Michigan	Chemical Engineering	Ph.D., 1984

(b) Appointments

2010 – present	Regents Professor
2008 – present	Distinguished Professor
2007 – present	Director of the Nanoscience & Microsystems graduate program, UNM
1994 – present	Director, Center for Micro-engineered Materials (CMEM)
2002 – 2007	Associate Chair, Department of Chemical and Nuclear Engineering
2004 – 2007	Site Director, NSF/IUCRC Ceramic and Composite Materials Center
1999 – 2004	Executive Director, NSF/IUCRC Ceramic and Composite Materials Cntr.
1994 – 1999	Director NSF/IUCRC Center for Microengineered Materials
1984 – present	Chemical & Nuclear Engineering, University of New Mexico
1976 – 1978	Hindustan Organic Chemicals, Rasayani, India, Scientific Officer
1975 – 1976	Hindustan Lever Ltd., Research Center, Bombay, India, Research Asst.

(c) Products

i. Five products most closely related to the proposed project

1. Q. Xu, K.C. Kharas, B.J. Croley, and A.K. Datye, *The Contribution of Alumina Phase Transformations to the Sintering of Pd Automotive Catalysts.* Topics in Catalysis, 2012. **55**(1-2): p. 78-83.
2. S.G. Wettstein, J.Q. Bond, D.M. Alonso, H.N. Pham, A.K. Datye, and J.A. Dumesic, *RuSn bimetallic catalysts for selective hydrogenation of levulinic acid to gamma-valerolactone.* Applied Catalysis B-Environmental, 2012. **117**: p. 321-329.
3. Z. Wei, J. Sun, Y. Li, A.K. Datye, and Y. Wang, *Bimetallic catalysts for hydrogen generation.* Chemical Society Reviews, 2012. **41**(24): p. 7994-8008.
4. B. Roy, U. Martinez, K. Loganathan, A.K. Datye, and C.A. Leclerc, *Effect of preparation methods on the performance of Ni/Al2O3 catalysts for aqueous-phase reforming of ethanol: Part I-catalytic activity.* International Journal of Hydrogen Energy, 2012. **37**(10): p. 8143-8153.
5. B. Roy, K. Artyushkova, H.N. Pham, L. Li, A.K. Datye, and C.A. Leclerc, *Effect of preparation method on the performance of the Ni/Al 2O3 catalysts for aqueous-phase reforming of ethanol: Part II-characterization.* International Journal of Hydrogen Energy, 2012. **37**(24): p. 18815-18826.

ii. Five other significant products

1. B. Roldan Cuenya, L.K. Ono, J.R. Croy, K. Paredis, A. Kara, H. Heinrich, J. Zhao, E.E. Alp, A.T. Delariva, A. Datye, E.A. Stach, and W. Keune, *Size-dependent evolution of the*

atomic vibrational density of states and thermodynamic properties of isolated Fe nanoparticles. Physical Review B - Condensed Matter and Materials Physics, 2012. **86**(16).
2. H.N. Pham, A.E. Anderson, R.L. Johnson, K. Schmidt-Rohr, and A.K. Datye, *Improved hydrothermal stability of mesoporous oxides for reactions in the aqueous phase.* Angewandte Chemie - International Edition, 2012. **51**(52): p. 13163-13167.
3. J. Matos, L.K. Ono, F. Behafarid, J.R. Croy, S. Mostafa, A.T. Delariva, A.K. Datye, A.I. Frenkel, and B. Roldan Cuenya, *In situ coarsening study of inverse micelle-prepared Pt nanoparticles supported on γ-Al 2O 3: Pretreatment and environmental effects.* Physical Chemistry Chemical Physics, 2012. **14**(32): p. 11457-11467.
4. B. Halevi, E.J. Peterson, A. Roy, A. Delariva, E. Jeroro, F. Gao, Y. Wang, J.M. Vohs, B. Kiefer, E. Kunkes, M. Hävecker, M. Behrens, R. Schlögl, and A.K. Datye, *Catalytic reactivity of face centered cubic PdZn α for the steam reforming of methanol.* Journal of Catalysis, 2012. **291**: p. 44-54.
5. A.D. Benavidez, L. Kovarik, A. Genc, N. Agrawal, E.M. Larsson, T.W. Hansen, A.M. Karim, and A.K. Datye, *Environmental transmission electron microscopy study of the origins of anomalous particle size distributions in supported metal catalysts.* ACS Catalysis, 2012. **2**(11): p. 2349-2356.

(d) Synergistic Activities

As director of a NSF/Research Experiences for Undergraduates Site Program, I have organized a summer program (since 1995) for students from other universities to spend 10 weeks on campus working with researchers at our center. During the summers of 1999- 2001, we also brought 3 high school teachers each year into our summer program via the RET (Research Experiences for Teachers) program funded by NSF. As the site director for the NSF/EPSCOR program in Nanoscience at UNM, I have helped organize an outreach program that involves workshops aimed at high school teachers. We secured funding from a foundation to provide kits that teachers can take back to their classes. We have developed a new interdisciplinary curriculum in Nanoscience and Microsystems, as part of the NSF/IGERT program.

(e) Collaborators & Other Affiliations

Collaborators (past 48 months)

Larry Allard, Jeff Brinker, Neil Coville, Bob Davis, Jim Dumesic, Paul Hansen, Stig Helveg, Charles Kappenstein, Karl C. C. Kharas, Chang Kim, Leon Lefferts, Jeff Miller, Matt Neurock, Robert Schløgl, Brent Shanks, John Vohs, Yong Wang

Graduate Advisor

Robert Lemlich – University of Cincinnati, Johannes Schwank – University of Michigan

Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

Students Graduated (27 Ph. D, 35 M. S) Recent graduates:
Patrick Burton, PhD 2011, Adam Tsosie, MS 2011, Elena Berliba Vera, MS 2011, Noel Dawson, MS 2011, Jim Fitch, MS 2012

Present Research group: Eric Petersen, Angelica Benavidez, Jonathan Paiz, Tyne Johns, Michael Stewart, Cristihan Carillo *undergrad students* Amanda Anderson, Aaron Jenkins, Johnny Nogales, Monique Padilla, Jay McCabe, Tyler Hough Current Postdoctoral Associates: Hien Pham, Andrew DeLaRiva, Haifeng Xiong, Sivakumar Challa

ROBERT J. DAVIS
Earnest Jackson Oglesby Professor, Department of Chemical Engineering
University of Virginia
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(a) Professional Preparation

Virginia Tech	Chemical Engineering	B.S., 1985
Stanford University	Chemical Engineering	M.S., 1987
Stanford University	Chemical Engineering	Ph.D., 1989
University of Namur, Belgium	Chemistry	Postdoc, 1989-1990

(b) Appointments

2009 – Present	Earnest Jackson Oglesby Professor, Chem. Engr., Univ. of Virginia
2002 – 2011	Professor and Chair, Chemical Engineering, University of Virginia
1996-2002	Associate Professor, Chemical Engineering, University of Virginia
1990-1996	Assistant Professor, Chemical Engineering, University of Virginia

(c) Products

i. Five products most closely related to the proposed project

1. S.E. Davis, M.S. Ide and R.J. Davis, "Selective Oxidation of Alcohols and Aldehydes over Supported Metal Nanoparticles," *Green Chem.* 15 (2013) 17-45.
2. M.S. Ide, B. Hao, M. Neurock and R.J. Davis, "Mechanistic Insights on the Hydrogenation of α,β-unsaturated Ketones and Aldehydes to Unsaturated Alcohols over Metal Catalysts," *ACS Catalysis* **2** (2012) 671-683.
3. S.E. Davis, B.N. Zope and R.J. Davis "On the mechanism of selective oxidation of 5-hydroxymethylfurfural to 2,5-furandicarboxylic acid over supported Pt and Au catalysts," *Green Chem.* **14** (2012) 143-147.
4. M. Chia, Y.J. Pagán-Torres, D. Hibbitts, Q. Tan, H.N. Pham, A.K. Datye, M. Neurock, R.J. Davis, and J.A. Dumesic, "Selective hydrogenolysis of polyols and cyclic ethers over bifunctional surface sites on rhodium-rhenium catalysts," *J. Am. Chem. Soc.* **133** (2011) 12675-12689.
5. B.N. Zope, D.D. Hibbitts, M. Neurock and R.J. Davis, "Reactivity of the Gold-Water Interface during Selective Oxidation Catalysis" *Science* **330** (2010) 74-78.

ii. Five other significant products

1. B.N. Zope and R.J. Davis, "Inhibition of Gold and Platinum Catalysts by Reactive Intermediates Produced in the Selective Oxidation of Alcohols in Liquid Water," *Green Chem.* **13** (2011) 3484-3491.
2. S.E. Davis, L.R. Houk, E.C. Tamargo, A.K. Datye, and R.J. Davis, "Oxidation of 5-Hydroxymethylfurfural over Supported Pt, Pd and Au Catalysts," *Catalysis Today* **160** (2011) 55-60.
3. O.M. Daniel, A. DeLaRiva, E.L. Kunkes, A.K. Datye, J.A. Dumesic and R.J. Davis, "X-ray Absorption Spectroscopy of Bimetallic PtRe Catalysts for Hydrogenolysis of Glycerol to Propanediols" *ChemCatChem* **2** (2010) 1107-1114.
4. E.P. Maris and R.J. Davis, "Hydrogenolysis of Glycerol over Carbon-supported Ru and Pt Catalysts," *J. Catal.* **249** (2007) 328-337.
5. W.C. Ketchie, Y.-L. Fang, M.S. Wong, M. Murayama, and R.J. Davis, "Influence of Gold Particle Size on the Aqueous-Phase Oxidation of Carbon Monoxide and Glycerol," *J. Catal.* **250** (2007) 94-101.

(d) Synergistic Activities

Chair of Gordon Conference on Catalysis, 2006.
Chair of Catalysis Programming of the AIChE, 2002-03 and Director of Catalysis and Reaction Engineering Division of AICHE, 2006-2008.
Chair of US Government panel conducting an international assessment of "Catalysis by Nanostructured Materials" 2007-2009.
Director-at-Large of the North American Catalysis Society, 2009-Present
Director of the ACS Catalysis Science and Technology Division, 2013-Present
Editorial Boards: Applied Catalysis A: General, 1998-2003; Applied Catalysis B: Environmental, 2004-2011; Journal of Molecular Catalysis A: Chemical, 2007-Present; ChemCatChem Heterogeneous, Homogeneous and Biocatalysis, 2009-2012; Journal of Catalysis, 2009-Present; ACS Catalysis, 2011-Present, Journal of Energy Chemistry, 2013-Present

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Pradeep Agrawal	Georgia Tech
Abhaya Datye	University of New Mexico
James Dumesic	University of Wisconsin
Chris Jones	Georgia Tech
M. Douglas Levan	Vanderbilt University
P.J. Ludovice	Georgia Tech
C.D. Sherrill	Georgia Tech
David Sholl	Georgia Tech
Marcus Weck	New York University
Michael Wong	Rice University
Daniela Ferrari	Dow Chemical Company
David Barton	Dow Chemical Company
Matthew Neurock	University of Virginia
Krijn de Jong	Utrecht University
Robert Schlögl	Fritz Haber Institute

ii. Graduate Advisors and Postdoctoral Sponsors

Michel Boudart	Stanford University
Eric Derouane	University of Namur

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

Joseph Kozlowski (Ph.D. 2013, UOP), Sara Davis (Ph.D. 2012, postdoc UVa), Bhushan Zope (Ph.D. – 2011, Applied Materials), Makarand Gogate (Postdoc – 2010, unknown), Yuanzhou Xi (Ph.D. – 2010, Postdoc – 2010, Cummins), Surbhi Jain (Ph.D. – 2008, ExxonMobil),

36 = Total number of graduate students advised and postdocs sponsored.

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(a) Professional Preparation

University of California, San Diego	Electrical Engineering	B.S., 1983
University of Southern California	Electrical Engineering	Ph.D., 1993

(b) Appointments

2012- present	Professor, Electrical & Computer Engineering, Iowa State Univ.
2011– present	Program Officer, National Science Foundation, BIO Directorate, Divition of Biological Infrastructure
2002 –present	Associate Professor, Electrical & Computer Engineering, Iowa State Univ.
1995 – 2002	Assistant Professor, Electrical & Computer Engineering, Iowa State Univ.
1994	Research Associate, Electrical Engineering, University of Southern California
1991 – 1993	Research Assistant, Electrical Engineering, University of Southern California
1988 – 1991	Senior Staff Engineer, Martin Marietta Space Systems
1983 – 1990	Member of the Technical Staff, Hughes Aircraft Corporation

(c) Products

i. Five products most closely related to the proposed project

1. Van Hemert, J.L., J.A. Dickerson, "Discriminating response groups in metabolic and regulatory pathway networks", Bioinformatics, *Bioinformatics*, 2012, doi: 10.1093/bioinformatics/bts039..
2. Fu, Y., L.R. Jarboe, J.A. Dickerson, "Reconstructing genome-wide regulatory network of *E. coli* using transcriptome data and predicted transcription factor activities," *BMC Bioinformatics,* **12**:233, 2011, doi:10.1186/1471-2105-12-233.
3. P. Bais, S.M. Moon-Quanbeck, B.J. Nikolau, J.A. Dickerson, "Plantmetabolomics.org: mass spectrometry-based Arabidopsis metabolomics," *Nucleic Acids Research,* 2012 Database Issue, Early Access November 11, 2011, doi: 10.1093/nar/gkr1006.
4. P Bais, SM. Moon, K He, R Leitao, K Dreher, T Walk, Y Sucaet, L Barkan, G Wohlgemuth, MR. Roth, ES Wurtele, P Dixon, O Fiehn, B.M Lange, V Shulaev, LW. Sumner, R Welti, BJ. Nikolau, S Rhee, and JA. Dickerson, "PlantMetabolomics.org: A Web Portal for Plant Metabolomics Experiments," *Plant Physiology*, pp. 109, **DOI:10.1104/pp.109.151027,** Feb. 10, 2010.
5. Xia, T, JS. Tong, SS Rathore, X Gu, JA Dickerson, "Network motif comparison rationalizes Sec1/Munc18-SNARE regulation mechanism in exocytosis," BMC Systems Biology, 6:19, 2012, doi: 10.1186/1752-0509-6-19.

ii. Five other significant products

1. Mao, L., Van Hemert*, J., Dash, S. and Dickerson, J. (2009) Arabidopsis gene co-expression network and its functional modules. *BMC Bioinformatics*, **10**, 346.
2. Grimplet, J., Cramer, G.R., Dickerson, J.A., Mathiason, K., Van Hemert, J. and Fennell, A.Y. (2009) VitisNet: "Omics" Integration through Grapevine Molecular Networks. *PLoS One*, **4**, e8365.
3. S.Y. Rhee, J.A. Dickerson, D. Xu, "Bioinformatics and Its Applications in Plant Biology," Annual Review of Plant Biology, **57**, 335-359, 2006.

4. Pan Du*, Jian Gong*, Eve Syrkin Wurtele, and J.A. Dickerson**,** "Modeling Gene Expression Networks using Fuzzy Logic," Special issue of *IEEE Transactions on Systems, Man and Cybernetics, Part B,* **35**(6):1351-1359, 2005.
5. Yuting Yang*, Levent Engin, Eve Syrkin Wurtele, Carolina Cruz-Neira, J.A. Dickerson, "Integration of metabolic networks and gene expression in virtual reality," *Bioinformatics*, **21**: 3645-3650.

(d) Synergistic Activities

Interdisciplinary Training in Bioinformatics: Mentored twenty IGERT fellows in lab rotations for computational biology, seven students were female; eight were domestic students. Mentored high school interns in computational biology, both students were female and underrepresented minorities.

Curriculum Development: Developed a new course on Systems Biology for the graduate program in Bioinformatics. Developed new sophomore-level course in signals and systems with labs featuring problems in computational biology.

Mentoring of underrepresented undergraduate students: Mentored two female, minority students (Alicia Guidry and Machele Lugo) Bioinformatics and Computational Biology NSF/NIH Summer Institute, 2005. Ms. Guidry is a MS student in CS at Texas A&M. Rien Beall, minority student for a project on graph-based clustering 2006. Mr. Beall graduated as a MS student at ISU in 2010.

Biology Education, grades 6-12: Developing the Meta!Blast video game for teaching plant cell biology and metabolism to middle and high school students. Meta!Blast features accurate models of cellular organelles and proteins from recent imaging studies and the protein databank (PDB).

Organization of Workshops or Special Courses (last 4 years): NIH/NSF Bioinformatics Summer Institute (2006-Present); 18th Annual GFST Symposium: Systems Biology: Integrative, Comparative, and Multi-Scale Modeling (2009).

(e) Collaborators & Other Affiliations

i. Collaborators (past 48 months)

D. Berleant,	Univ. Arkansas	D Reiners	Univ. of Louisiana
G. Cramer	Univ. Nevada Reno	SY Rhee	The Carnegie Institute
C. Cruz-Neira	Univ. of Louisiana	K.Y. San	Rice
O. Feihn	UC Davis	V. Shulaev	Virginia Tech
A. Fennell	South Dakota State Univ.	L Sumner	Noble Institute
R. Gonzalez	Rice	D. Xu	Univ. of Missouri-Columbia
M. Lange	University of Washington	R. Welti	Kansas State Univ.
D. Marshall	Scottish Crop Res. Institute		

ii. Graduate and Postdoctoral Advisors

Bart Kosko, Univ. of Southern California, Petros Ioaunnou, Univ. of Southern California

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

Past Students

Sudhansu Dash (Iowa State Univ.), Pan Du (Northwestern Univ.), Joset Etzel (Univ. Medical Center Groningen, Netherlands, Caltech), Shubha Kher (Arkansas State Univ.), Linyong Mao (Iowa State Univ.), Yuting Yang (Intel), Ming Jia (Google), Preeti Bais (Stemina), Wengang Zhou and John Van Hemert (Pioneer)

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University of Wisconsin
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(a) Professional Preparation

University of Wisconsin	Chemical Engineering	B.S., 1971
Stanford University	Chemical Engineering	M.S., 1972
Stanford University	Chemical Engineering	Ph.D., 1974

(b) Appointments

1/96 - present	Steenbock Professor, Chemical Engineering Department, University of Wisconsin (UW) – Madison
1/98 - 7/00	Chair, Chemical Engineering Department, UW – Madison
1/89 - 1996	Milton and Maude Shoemaker Professor, UW – Madison
1/93 - 7/95	Chair, Chemical Engineering Department, UW – Madison
9/92 -12/92	Acting Chair, Chemical Engineering Department, UW – Madison
6/89 - 9/92	Associate Chair, Chemical Engineering Department, UW – Madison
6/82 - 12/88	Professor, Chemical Engineering, UW – Madison
6/79 - 6/82	Associate Professor, Chemical Engineering, UW – Madison
1/76 - 6/79	Assistant Professor, Chemical Engineering, UW – Madison

(c) Products (~370 total)

i. Five products most closely related to the proposed project

1. Bifunctional Solid Catalysts for the Selective Conversion of Fructose to 5-Hydroxymethylfurfural, *Topics in Catalysis* **53**, 1185 (2010), A. J. Crisci, M. H. Tucker, S. L. Scott, and J. A. Dumesic.
2. Production of 2,5-dimethylfuran from biomass-derived carbohydrates for liquid transportation fuels, *Nature* **447**, 982 (2007), Y. Román-Leshkov, C. J. Barrett, Z. Y. Liu, and J. A. Dumesic.
3. Catalytic Conversion of Biomass to Biofuels, *Green Chemistry* **12**, 1493 (2010), Jesse Q. Bond, David Martin Alonzo, and J. A. Dumesic.
4. Selective hydrogenolysis of polyols and cyclic ethers over bifunctional surface sites on rhodium-rhenium catalysts, *Journal of the American Chemical Society* **133**, 12675 (2011), Mei Chia, Yomaira J. Pagán-Torres, David Hibbitts, Qiaohua Tan, Hien N. Pham, Abhaya K. Datye, Matthew Neurock, Robert J. Davis, and J. A. Dumesic.
5. Integrated catalytic system to convert γ-valerolactone to liquid alkenes for transportation fuels, *Science* **327**, 1110 (2010), J. Q. Bond, D. Martin-Alonso, R. M. West, D. Wang and J. A. Dumesic.

ii. Five other significant products

1. Inter-conversion between γ-valerolactone and pentenoic acid combined with decarboxylation to form butene over silica/alumina, *Journal of Catalysis* **281**, 290 (2011), Jesse Q. Bond, Dong Wang, David Martin Alonso, and J. A. Dumesic.

2. Production of Biofuels from Cellulose and Corn Stover Using Alkylphenol Solvents, *Chemistry and Sustainability* **4**, 1078 (2011), David Martin Alonso, Stephanie G. Wettstein, Jesse Q. Bond, Thatcher W. Root and James A. Dumesic.
3. Production of liquid hydrocarbon fuels by catalytic conversion of biomass-derived levulinic acid, *Green Chemistry* **7**, 1755 (2011), Drew J. Braden, Carlos A. Henao, Jacob Heltzel, Christos C. Maravelias and James A. Dumesic.
4. Liquid-phase catalytic transfer hydrogenation and cyclization of levulinic acid and its esters to γ-valerolactone over metal oxide catalysts, *Chemical Communications* 47(44), 12233 (2011), Mei Chia and James A. Dumesic.
5. Synthesis of highly ordered hydrothermally stable mesoporous niobia catalysts by atomic layer deposition, *ACS Catalysis* **1**, 1234 (2011), Yomaira J. Pagán-Torres, Jean Marcel R. Gallo, Dong Wang, Hien N. Pham, Joseph A. Libera, Christopher L. Marshall, Jeffrey W. Elam, Abhaya K. Datye, and James A. Dumesic.

(d) Synergistic Activities

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

ii. Graduate Advisors and Postdoctoral Sponsors

Michael Boudart, Standford University

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)*

Yomaira Pagan	September 2011	Dow Chemical
Mark Tucker	June 2011	BP
Jesse Bond*	July 2011	Syracuse University
Drew Braden	August 2010	BP
Juan Carlos Serrano-Ruiz*	October 2009	University of Córdoba
Ed Kunkes	August 2009	Fritz Haber Institute
Ryan West	June 2009	Proctor and Gamble
Chris Barrett	August 2008	General Foods
Yuriy Roman-Leshkov	August 2008	MIT
Dante Simonetti	July 2008	UOP

14 = total number of graduate students advised and postdocs sponsored.

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Depts. Chemical & Biomolecular Eng. and Bioengineering
Rice University, Houston, TX 77005
(713) 348-4893/(713) 348- 5478 (fax)/ramon.gonzalez@rice.edu

(a) Professional Preparation

Central University of Las Villas, Cuba	Chemical Engineering	B.Sc., 1993
Catholic University of Valparaiso, Chile	Biochemical Engineering	M.Sc., 1999
University of Chile, Chile	Chemical Engineering	Ph.D., 2001
University of Florida	Microbiology &Cell Science	PostDoc, 2001-2002

(b) Appointments

2011–Present	Associate Professor, Dept. Chemical & Biomolecular Eng., Dept. Bioengineering, Rice University, Houston, Texas.
2005–2011	William W. Akers Assistant Professor, Dept. Chemical & Biomolecular Eng., Dept. Bioengineering, Rice University, Houston, Texas.
2002–2005	Assistant Professor, Depts. of Chemical & Biological Eng. and Food Science & Human Nutrition, Iowa State University, Ames, Iowa.
2001–2002	Postdoctoral Associate, Dept. Microbiology & Cell Science, Univ. of Florida, Gainesville, Florida.
1994–1995	Process Engineer , Marcelo Salado Sugar Mill (Formerly, Reforma Sugar Mill), MINAZ (Cuba's Sugar Ministry), Caibarien, Cuba.
1993–1996	Research Associate and Lecturer, Center for Processes Analysis, Dept. Chemical Eng., Central University of Las Villas, Santa Clara, Cuba.

‘

(c) Products

i. Five products most closely related to the proposed project

1. Blankschien, M. D., Pretzer, L.A., Huschka, R., Halas, N.J., Gonzalez, R., and Wong, M.S. (2013). Light-Triggered Biocatalysis Using Thermophilic Enzyme–Gold Nanoparticle Complexes. ACS Nano 7(1): 654-663.
2. Mazumdar, S., Blankschien, M. D., Clomburg, J. M., and Gonzalez, R. (2013). Efficient synthesis of L-lactic acid from glycerol by metabolically engineered Escherichia coli. Microb. Cell Fact. 12: 7.
3. Clomburg, J.M., and Gonzalez, R. (2013). Anaerobic fermentation of glycerol: a platform for renewable fuels and chemicals. Trends Biotechnol. 31 (1), 20-28.
4. Rastogi G., Gurram R., Bhalla A., Gonzalez R., Bischoff K., Hughes S., Kumar S., and Sani R.K. (2013). Presence of glucose, xylose, and glycerol fermenting bacteria in the deep biosphere of the former Homestake gold mine, South Dakota. Front. Microbio. 4:18.
5. Clomburg, J.M., Vick, J.E., Blankschien, M. D., Rodriguez-Moya, M., Gonzalez, R. (2012). A synthetic biology approach to engineer a functional reversal of the β-oxidation cycle. ACS Synth. Biol. 1 (11), 541-554.

ii. Five other significant products

1. Park, J., Rodriguez-Moya, M., Li, M., Pichersky, E., San, K-Y., and Gonzalez, R. (2012). Synthesis of methyl ketones by metabolically engineered Escherichia coli. J. Industrial Microbiol. Biotechnol. 39 (11), 1703-1712.
2. Posada, J. A., Cardona, C. A., Gonzalez, R. (2012). Analysis of the production process of optically pure D-lactic acid from raw glycerol using engineered Escherichia coli strains. Appl. Bioch.

Biotechnol. 166 (3): 680-699.
3. Cintolesi, A., Clomburg, J.M., Rigou, V., Zygourakis, K., and Gonzalez, R. (2012). Quantitative analysis of the fermentative metabolism of glycerol in Escherichia coli. Biotechnol. Bioeng. 109 (1): 187-198.
4. Dellomonaco, C., Clomburg, J.M., Miller, E.N., and Gonzalez, R. (2011). Engineered reversal of the β-oxidation cycle for the synthesis of fuels and chemicals. Nature 476, 355-359.
5. Clomburg, J., and Gonzalez, R. (2011). Metabolic engineering of Escherichia coli for the production of 1,2-propanediol from glycerol. Biotechnol. Bioeng. 108 (4): 867-879.

(d) Synergistic Activities

- Senior Editor: Journal of Industrial Microbiology and Biotechnology.
- Editorial Board: Applied and Environmental Microbiology; Applied Biochemistry and Biotechnology; Food Biotechnology.
- Program Chair, 2011 Society for Industrial Microbiology Annual Meeting, July 2011, New Orleans, LA.
- Organized and Chaired Sessions on Metabolic Engineering for Biofuels/Biorenewables in AIChE, ACS, and Rice's Institute for Biosystems and Bioengineering meetings.
- Organized and Co-Chaired "Microbial Science and Technology" session, Symposium on Biotechnology for Fuels and Chemicals, San Francisco, CA, 2009.
- Co-Chaired "Rational design in Metabolic Engineering" Session in the Metabolic Engineering V Conference, Lake Tahoe, CA, 2004.
- Participant in DOE workshop on "Biomass to Biofuels" (December 2005), which resulted in a Roadmap for Developing Cleaner Fuels (Report: "Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda").
- Ad hoc and panel reviewer for: NSF, USDA, DOE, NIH, and FONDECYT (National Foundation for Science and Technology, Chile).
- Ad hoc reviewer for: Proceedings of the National Academy of Sciences of the USA; Metabolic Engineering; Biotechnology and Bioengineering; Applied and Environmental Microbiology; Applied Microbiology and Biotechnology; Applied Biochemistry and Biotechnology; Genome Research; Molecular Systems Biology; Biophysical Journal; Biotechnology Progress; Food Biotechnology.

(e) Collaborators & Other Affiliations

i. Collaborators

D. Ramkrishna (Purdue University); K.Y. San, M. Wong & P. J. J. Alvarez (Rice University); J. V. Shanks & T. Bobik (Iowa Sate University); C. Altamirano & J. Berrios (Catholic University of Valparaiso, Chile).

ii. Advisor and Postdoctoral Sponsors

***Postdoctoral*:** Prof. Lonnie O. Ingram (Postdoctoral, University of Florida)

***Graduate*:** Prof. Juan A. Asenjo and B. A. Andrews (Ph.D., University of Chile) and Prof. J. C. Gentina (MSc., Catholic University of Valparaiso)

iii. Serving/Served as Advisor (at Rice University and Iowa State University)

- Graduate students: Y. Dharmadi, A. Murarka, A. Gupta, R. San, V. Rigou, J. Clomburg, G. Durnin, C. Dellomonaco, A. Cintolesi, M. Rodriguez-Moya, J. Park, S. Young, S. Kim, S. Dash
- Undergraduate students: T. Pritchard-Meaker, J. Penley, J. Rixen, S. Hothman, M. Preacher, K. Smith, M. Tobelmann, T. Schiling, B. Shite, M. Yu, J. Ross, Z. Yeates, V. Solorzano, K. Wilson, T. Hyunh, J. Miller
- High school students: W. Dewing, O. Quintero, C. Thomas, P. de Guzman, J. Chapman.
- PostDocs: J. Vick, J. Clomburg, E. Miller, M. Blankschien, S. Mazumdar, S. Yazdani

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Iowa State University
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(a) Professional Preparation

University of Kentucky	Chemical Engineering	B.S., 2000
University of California, Los Angeles	Chemical and Biomolecular Engineering	Ph.D., 2006
University of Florida	Florida Center for Renewable Fuels and Chemicals	Postdoctoral Researcher, 2006-2008

(b) Appointments

2012 current member of Toxicology Interdepartmental Program, ISU
2010-current Affiliate member of Bioinformatics & Computational Biology Program, ISU
2008-current member of Interdepartmental Microbilogy Program, ISU
2008-current Assistant Professor, Chemical and Biological Engineering, Iowa State University

(c) Products

i. Five products most closely related to this work

1. P. Liu, A. Chernyshov, T. Najdi, Y. Fu, J. Dickerson, S. Sandmeyer, **L. Jarboe*** "Membrane stress caused by octanoic acid in *Saccharomyces cerevisiae*" Applied Microbiology and Biotechnology 2013 (*in press*)
2. P. Liu, **L. Jarboe*** "Metabolic Engineering of biocatalysts for carboxylic acids production" Computational and Structural Biology 2012. 3(4) e201210011, http://dx.doi.org/10.5936/csbj.201210011
3. L.A. Royce, E. Boggess, T. Jin, J. Dickerson, **L. Jarboe***. "Identification of Mutations in Evolved Bacterial Genomes" *Methods in Molecular Biology* (vol 985): *Systems Metabolic Engineering: Methods and Protocols*, Hal Alper (ed). 2013 (*in press*)
4. **L. R. Jarboe***, Ping Liu, Liam Royce "Engineering inhibitor tolerance for the production of biorenewable fuels and chemicals". *Current Opinion in Chemical Engineering*. 2011 (invited) 1:38-42. DOI 10.1016/j.coche.2011.08.003
5. Yao Fu, **Laura R. Jarboe**, Julie Dickerson*. "Reconstructing genome-wide regulatory network of *E. coli* using transcriptome data and predicted transcription factor activities". *BMC Bioinformatics*. 2011 12:233.

ii. Other significant products

1. **L. Jarboe***, P. Liu, K. Kautharapu, L.O. Ingram "Optimization of enzyme parameters for fermentative production of biorenewable fuels and chemicals" Computational and Structural Biotechnology Journal. October 2012. 3(4) e201210005, http://dx.doi.org/10.5936/csbj.201210005
2. K.B. Kautharapu, **L. Jarboe***, "Genome sequence of psychrophilic deep sea bacterium *Moritella marina* MP-1" Journal of Bacteriology, November 2012. 194:6296-6297. http://jb.asm.org/cgi/content/abstract/194/22/6296?etoc
3. K. B. Kautharapu, J. Rathmacher, **L. Jarboe*** "Growth condition optimization for docosahexaenoic acid (DHA) production by *Moritella marina* MP-1" 2012 Applied Microbiology and Biotechnology DOI: 10.1007/s00253-012-4529-7 http://www.springerlink.com/content/n37573r377502523/

4. Donovan Layton, A. Ajjarapu, D.W. Choi, **L. Jarboe***. "Engineering ethanologenic *Escherichia coli* for levoglucosan utilization". *Bioresource Technology*. 2011 102:8318-8322.
5. **L. R. Jarboe*,** Z. Wen, D.W. Choi, R.C. Brown "Hybrid thermochemical processing: fermentation of pyrolysis-derived bio-oil". *Applied Microbiology and Biotechnology*. 2011 (invited) 91(6):1519-1523.

(d) Synergistic Activities

- Designed and implemented a fermentation demonstration module for CBiRC RET program, K12 students (2009, 2012, 2013)
- Research experience for Undergraduates, Young Engineers and K-12 Teachers: ISU students (30), REU students (8), Young Engineers (2), RET (2)
- Session (co-)chair for Society for Industrial Microbiology (2009, 2010, 2011, 2012), American Institute of Chemical Engineers (2009, 2010, 2011, 2012), Frontiers in Biorefining (2012)
- Reviewer for *Biotechnology Progress*, *Journal of Theoretical Biology*, *Journal of Biomedicine and Biotechnology, Biotechnology and Bioengineering, Metabolic Engineering, Bioresource Technology, PLoS ONE, Proteome Science*
- Review panel: NIH SBIR/STTR: 3/2011, 7/2011, 11/2011, 3/2012, 7/2012; NSF BIO: 1/2012, 2/2013; NSF CBET: 5/2012.

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Srinivas Aluru, Iowa State University	Ka-Yiu San, Rice University
Robert Brown, Iowa State University	Suzanne Sandmeyer, Univ. of California, Irvine
Nancy DaSilva, University of California, Irvine	Jackie Shanks, Iowa State University
Julie Dickerson, Iowa State University	K. T. Shanmugam, University of Florida
Ramon Gonzalez, Rice University	Zengyi Shao, Iowa State University
Cathy Logue, Iowa State University	Michelle Soupir, Iowa State University
David Nielsen, Arizona State University	Martin Spalding, Iowa State University
Lisa Nolan, Iowa State University	Michael Thompson, Iowa State University
D. Raj Raman, Iowa State University	Xhiyou Wen, Iowa State University
John Rathmacher, Metabolic Technologies, Inc	Mark Wright, Iowa State University
Derrick Rollins, Iowa State University	Olga Zabotina, Iowa State University

ii. Graduate Advisors and Postdoctoral Sponsors

James C. Liao	University of California, Los Angeles
Lonnie O. Ingram	University of Florida

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Graduate Students:	
Tao Jin	Iowa State University, Chemical and Biological Engineering
Chunyu Liao	Iowa State University, Microbiology
Ping Liu	Iowa State University, Microbiology
Liam Royce	Iowa State University, Chemical and Biological Engineering
Martha Zwonitzer	Environmental Sciences
Postdoctoral Researchers:	
Zhanyou Chi	Iowa State University, Chemical and Biological Engineering
Kumar B. Kautharapu	Iowa State University, Chemical and Biological Engineering

7 = Total number of graduate students advised and postdocs sponsored.

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(a) Professional Preparation

Hertfordshire University, UK	Applied Biology	B.S., 1972 - 1976
Council Nat Academic Awards, UK	Biochemistry and Toxicology	Ph.D., 1976 - 1981
ICI/Zeneca Seeds, Cheshire, UK	Biochemistry	Postdoc., 1981 – 1984

(b) Appointments

2009 – present	Director, Innovation Program, CBiRC, Iowa State University, Ames, IA
2007 – present	Founder/Co-Founder, EnaGen, GlucanBio, OmegaChea, SusTerea, Ames, IA
2000 – 2007	Unit R&D Director, BASF Plant Science, ExSeed Research, Ames, IA.
1994 – 2000	Founder and Research Director, ExSeed Genetics LLC., Ames, IA.
1988 – 1994	Group Manager, ICI/Zeneca Seeds, Biochem, Physiol & Genetics, Slater, IA.
1981 – 1988	Project Leader, ICI/Zeneca Seeds, Grain Filling, Runcorn, Cheshire, UK

(c) Products

Products Most Closely Related to the Proposed Project

1. Keeling, P.L. and Myers, A.M. Biochemistry and Genetics of Starch Synthesis, *Annual Review of Food Science and Technology, Annual Reviews.* **1** 271-303 (2010)
2. Hennen-Bierwagen TA, Lin Q, Grimaud F, Planchot V, Keeling PL, James MG, Myers AM. Proteins from Multiple Metabolic Pathways Associate with Starch Biosynthetic Enzymes in High Molecular Weight Complexes: A Model for Regulation of Carbon Allocation in Maize Amyloplasts. *Plant Physiology*. **146** 1541-1559 (2009).
3. Klucinec J.D. and Keeling P.L. Genetic modifications of plant starches for food applications. *In: Functional Foods & Biotechnology* (Vol 165, Food Science and Technology). Ed: Shetty, Paliyath, Pometto and Levin. Taylor & Francis, New York. (2006).
4. Tziotis A, Seetharaman K, Klucinec JD, Keeling P, White PJ. Functional properties of starch from normal and mutant corn genotypes. *Carbohydrate Polymers*: **61**: 238-247. (2005).
5. Gao Z., Guan H. and Keeling P.L. Involvement of lysine-193 of the conserved "KTGG" motif in the catalysis of maize starch synthase IIa. *Annals Biochemistry and Biophysics*, **427**: 1-7. (2004)

Other Significant Products

1. Multiple Patents/Patent Applications: (1) *US20050241020* (BASF) Transgenic Corn having Enhanced Nutritional Qualities; (2) *WO9902668* (BASF) Animal feed with low phytic acid, oil burdened and protein laden grain; (3) *US6881433* (BASF) Food products containing altered starch; (4) *WO200333540* (BASF) Starch; (5) *WO9815621* (ExSeed Genetics) Waxy wheat starch types having waxy proteins in granule; (6) WO9814601 (ExSeed Genetics) Encapsulation of polypeptides within the starch matrix; (7) *WO200279410* (ExSeed Genetics) Glucan chain length domains; (8) *WO9844780* (ExSeed Genetics) Plant like starches and the method of making them in hosts; (9) *WO9720936* (ExSeed Genetics) Modification of starch synthesis in plants; (10) *US6218155* (ExSeed Genetics) Plants and processes for obtaining them; (11) *WO9907211* (ExSeed Genetics) Controlled germination using inducible phytate gene; (12) *WO9848610* (ExSeed Genetics) Method of making

amylose mutant inbreds and hybrids; (13) *US5792920* (ICI/Zeneca) Plants with altered ability to synthesize starch & process for obtaining them; (14) WO9411520 (Zeneca/Syngenta) Novel plants and processes for obtaining them; (15) WO9409144 (Zeneca/Syngenta) Novel plants and processes for obtaining them; (16) WO9404693 (Zeneca/Syngenta) Novel plants and processes for obtaining them; (17) EP664835 (Zeneca/Syngenta) Novel plants and processes for obtaining them; (18) WO9535026 (Zeneca/Syngenta) Novel plants and processes for obtaining them.

(d) Synergistic Activities

2011 – present	Instructor for "Entrepreneurship" course (BR C 507X) as part of CBiRC Graduate Minor program. Directing the startup mentoring program in Biobased Foundry.
2009 – present	Multiple presentations at BioBased Summits, Bio World Congress meetings and Food Science Meetings
2007 – present	Multiple grant proposal submissions to USDA/NSF as PI/Co-PI with Drs. Alan Myers and Martha James, ISU, Biochem, Biophys & Molec Biology Dept, Ames, IA
1994 – present	Member of the ISU Interdepartmental Major in Plant Biology and POS Committee PhD and Masters Students as Affiliate Professor, Dept. Agronomy, ISU, Ames, IA
1988 – 2009	Board Member, ExSeed Genetics and BASF Plant Science, Ames, IA and Limburgerhof, Germany

(e) Collaborators & Other Affiliations

Collaborators & Co-Editors (past 48 months)

Bryant, Jonathan	BASF Corporation, RTP, NC
Denyer, Kay	The John Innes Center, UK
Dumesic, James	University of Wisconsin, Madison, WI
Guan, Hanping	BASF Corporation, RTP, NC
James, Martha	Iowa State University, Ames, IA
Klucinec, Jeff	BASF Corporation, Ames, IA
Kraus, George	Iowa State University, Ames, IA
Logemann, Juergen	BASF Corporation, Limburgerhof, Germany
Myers, Alan	Iowa State University, Ames, IA
Nikolau, Basil	Iowa State University, Ames, IA
Shanks, Brent	Iowa State University, Ames, IA
Smith, Alison	The John Innes Center, UK

Graduate Advisors and Postdoctoral Sponsors (my own)

Aldridge, Norman	MRC Toxicology Unit, Carshalton, Surrey, UK
Bridges, Ian	Syngenta, United Kingdom
Smith, Lewis	Astra Zeneca, United Kingdom

Thesis Advisor and Postgraduate-Scholar Sponsor

Miller, Rachel	Iowa State University, Ames, Iowa
1	= Total number of graduate students advised and postdocs sponsored

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(a) Professional Preparation

University of Rochester	Chemistry	B.S., 1972
Columbia University	Chemistry	Ph.D., 1976

(b) Appointments

2004	University Professor
1993-1999	Chair of Chemistry, Iowa State University
1986	Professor of Chemistry, Iowa State University
1981-1986	Associate Professor of Chemistry, Iowa State University
1976	Assistant Professor of Chemistry, Iowa State University

(c) Products

Products Most Closely Related to the Proposed Project

1. G. A. Kraus, T. Guney "A Direct Synthesis of 5-Alkoxymethylfurfural Ethers from Fructose", *Green Chemistry*, **2012**, 14, 1593-1596.
2. G. A. Kraus, S. Riley, T. Cordes "Aromatics from Pyrones: Para-Substituted Alkyl Benzoates from Alkenes and Coumalic Acid and Methyl Coumalate" *Green Chem*. **2011**, 2734-2736.
3. G. A. Kraus, H. Guo "One-Pot Synthesis of 2-Substituted Indoles from 2-Aminobenzyl Phosphonium Salts. A Formal Total Synthesis of Arcyriacyanin A," *Org. Lett*, **2008**, *10*, 3061-63.
4. G. A. Kraus, I. Jeon "Progress towards the synthesis of Papuaforin A: Selective formation of α- bromoenones from silyl enol ethers," *Tetrahedron Letters*, **2008**, *49*, 286-8.
5. G. A. Kraus, I. Jeon "Use of Allylic Strain to Enforce Stereochemistry.A Direct Synthesis of Calamenenes from *Hypericum elodeoides*," *Organic Letters*, **2006**, *8*, 5315-6316.

Other Significant Products

1. G. A. Kraus, H. Guo "A Flexible Synthesis of 2,3-Disubstituted Indoles fromAminobenzyl Phosphoniun Salts. A Direct Synthesis of Rutaecarpine" *J. Org. Chem*. **2009**, *74*, 5337.
2. G. A. Kraus, S. Roy "First Total Synthesis of Chrysosplenol D", *J. Natural Prod*. **2008**, *71 (11)*, 1961.
3. G. A. Kraus "Synthetic Methods for the Preparation of 1,3-Propanediol" *Clean Soil, Air, Water* (invited review), **2008**, *36*, 648-651.
4. G. A. Kraus, T. Wu "A Concise Synthesis of 5-Demethyl-HKI 0231A and 5-Demethyl-HKI 0231B," *Tetrahedron Letters*, **2006**, *47*, 7801.
5. G. A. Kraus, J. Kim "Synthesis of the Tetracyclic Ring System of Cumbiasin Via Tandem Radical Cyclizations," *Tetrahedron Letters*, **2006**, *47*, 7797.

(d) Synergistic Activities

- Director of the Institute for Physical Research and Technology
- Participant in NSF ERC
- PI of successful DOE grant "Development of A Biobased Graduate Minor"
- College of Liberal Arts and Sciences Award for Excellence in Research/Artistic Creativity (2001)
- Co-PI of NIH Center grant to examine botanical dietary supplements

(e) Collaborators & Other Affiliations

Collaborators & Co-Editors

Dr. Greg Phillips, Iowa State University
Dr. Marit Nilsen-Hamilton, Iowa State University
Dr. Anumantha Kanthasamy, Iowa State University
Dr. James Dumesic, Wisconsin
Dr. Brent Shanks, Iowa State University

Graduate Advisors and Postdoctoral Sponsors (my own)

Gilbert Stork, Columbia University

Thesis Advisor and Postgraduate-Scholar Sponsor

Total Postdoctoral Fellows advised: 25
Total Ph.D. students advised: 47
Total M.S. students advised: 12
Present graduate students: 9

Postdoctoral fellows in last 5 years

Dr. Yi Yuan 2004-2008
Dr. Sudipta Roy 2006-2007

Graduate students in last 5 years

Ph.D. students:

Vinayak Gupta 2010 (Scripps)
Insik Jeon 2006 (Columbia)
Jaehoon Bae 2006 (ConocoPhillips)
Yeung-Ho Seo 2006 (Michigan)
Tao Wu 2006 (Novartis Genomics Institute)
Jingqiang Wei 2005 (Broad Institute)

M.S. students:

Aniket Thite 2007
Tino Goronga 2007
Jacob Schroeder 2005

Adah Leshem
Pre-College Education Director
NSF Engineering Research Center for Biorenewable Chemicals
Iowa State University
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(a) Professional Preparation

King's College, University of London, UK	Environmental Science	B.Sc. 1980
University of Cambridge, UK	Applied Biology	M. Phil. 1981
Tel-Aviv University, Israel	Environment Physiology	Ph.D. 1989

(b) Appointments

2003 –	**Program Director**, Pre-College Education Outreach, Iowa State University
2000 - 2002	**Adjunct Assistant Professor**, Department of Zoology and Genetics, Iowa State University
1997 –2002	**Program Coordinator**, International Institute of Theoretical and Applied Physics Iowa State University
1995 - 1997	**Advising Coordinator**, Biology Program, Iowa State University
1986 - 1992	**Temporary Assistant Professor**, Department of Zoology and Genetics, Iowa State University

(c) Products

1. P. Hondred,b K. M. Haen, A. Leshem, and M. R. Kessler "Iowa State University Symbi GK12 Program: A Case Study of the Resident Engineer's Effect on 8th Graders Attitudes Toward Science and Engineering" Proceedings of the 2012 ASEE Annual Conference, June 10-13, 2012. San Antonio, TX. AC 2012-3027. Pages 9.

(d) Synergistic Activities

1. Director of Pre-College Education, NSF Engineering Research Center for Biorenewable Chemicals. (http://www.cbirc.iastate.edu/precollege.asp)
2. Director, Research Opportunities in Molecular Biology, Biotechnology and Genomics, a summer research experience program for middle/high school biology teachers (RET) that includes molecular biotechnology and genomics theory and technique training, curriculum and instruction development and a six week research component. (http://www.eeob.iastate.edu/plantgenomeoutreach)
3. Director, Iowa GK-12 Symbi Program. This NSF funded program is a partnership between Iowa State University and the Des Moines, IA, Public School District to support Ph. D. candidates in STEM fields develop strong communication skills by serving a resident scientists/engineers in K-12 classrooms. (http://www.gk12.iastate.edu/default.asp)
4. Director, "Partnerships for Research Education in Plants" in Iowa. This program provides genuine research experiences to over 300 high school students in Iowa as well as teachers, while helping scientists to discover the function of previously uncharacterized plant genes. (http://www.prep.biotech.vt.edu/index.html)
5. Director, Young Engineers and Scientists at Iowa State University, which provides high school students with a semester long, extracurricular, research experience under the mentorship of

science and engineering faculty at Iowa State University.
(http://www.plantgenomeoutreach.eeob.iastate.edu/HSS.htm)

(e) Collaborators & Other Affiliations

i. Collaborators
Adam Bogdanove, Cornell University; Anne Bronikowski, Iowa State University; Dawn Del Carlo, University of Northern Iowa; Drena Dobbs, Iowa State University; Erin Dolan, Virgina Polytechnic Institute and State University; Laura Jarboe, Iowa State University; Mari Kemis, Iowa State University; Basil Nikolau, Iowa State University; Thomas Peterson, Iowa State University; Raj Raman, Iowa State University; Brent Shanks, Iowa State University; Michelle Soupir, Iowa State University; Martin Spalding, Iowa State University; Jay Staker, Iowa State University; Amy Toth, Iowa State University; Dan Voytas, University of Minnesota; Jonathan Wendel, Iowa State University; Roger Wise, Iowa State University; Steve Whitham, Iowa State University; Eve Wurtele, Iowa State University; Yanhai Yin, Iowa State University

ii. Graduate Advisors and Postdoctoral Sponsors
Amos Ar, Tel-Aviv University Israel, Ralph Ackerman, Iowa State University

iii. Thesis Advisor and Postgraduate-Scholar Sponsor
N/A

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(a) Professional Preparation

1990-1995	Princeton University, Princeton, New Jersey Ph.D. in Chemical Engineering, May 1995 M.A. in Chemical Engineering, June 1992
1985-1990	Aristotle University, Thessaloniki, Greece Diploma in Chemical Engineering, June 1990

(b) Appointments

Jun. 2004-present	The Pennsylvania State University, Department of Chemical Engineering Professor
June 2005-present	Donald B. Broughton Professor in Chemical Engineering
May 2005-present	Director of Graduate Student Recruiting for the Bioinformatics & Genomics Option
Jan. 2004-present	Faculty Affiliate of the Center for Supply Chain Research
Sep. 2001-present	Member of Faculty of the Intercollege Graduate Degree in Integrative Biosciences (Bioinformatics & Genomics Option)
Jun. 2001-June 2004	The Pennsylvania State University, Department of Chemical Engineering Associate Professor
Sep. 1997-present	Member of Faculty of the Operations Research Program
Sep. 1995-Jun. 2001	The Pennsylvania State University, Department of Chemical Engineering Assistant Professor
Sept. 1990.-May 1995.	Princeton University, Dept. of Chemical Engineering Research Assistant
Feb.-Jul. 1990	Chemical Process Research Institute (CPERI), Greece Research Assistant

(c) Products

i. Five products most closely related to the proposed project

1. Zomorrodi A.R., P.F. Suthers, S. Ranganathan and C.D. Maranas (2012), "Mathematical optimization applications in metabolic networks," *Metabolic Engineering*, Vol. 14, 672-686.
2. Ranganathan, S., T.W. Tee, A. Chowdhury, A.R. Zomorrodi, J.M. Yoon, Y. Fu, J.S. Shanks, C.D. Maranas (2012), "An integrated computational and experimental study for overproducing fatty acids in *Escherichia coli*," *Metabolic Engineering*, Vol. 14, 687-704.
3. Zomorrodi, A.R. and C.D. Maranas (2012), "OptCom: A Multi-Level Optimization Framework for the Metabolic Modeling and Analysis of Microbial Communities," *PLoS Comput. Biol.,* Vol. 8, Issue 2, e1002363.
4. Kumar, A., P. Suthers and C.D. Maranas (2012), "MetRxn: A Knowledgebase of Metabolites and Reactions Spanning Metabolic Models and Databases," BMC Bioinformatics, Vol. 13, Issue 6, doi:10.1186/1471-2105-13-6.
5. Ranganathan, S., P.F. Suthers and C.D. Maranas (2010), "OptForce: An Optimization Procedure for Identifying All Genetic Manipulations Leading to Targeted Overproductions," *PLoS Comput. Biol*., Vol. 6, No. 4, e1000744.

ii. Five other significant products

1. Suthers, P.F., M.S. Dasika, V.S. Kumar, G. Denisov, J.I. Glass, and C.D. Maranas (2009), "A genome-scale metabolic reconstruction of Mycoplasma genitalium iPS189," *PLoS Comput. Biol., Vol. 5*, No. 2, e1000285.
2. Cheng, Y.J., P.F. Suthers, and C.D. Maranas (2008), "Identification of Optimal Measurement Sets for Complete Flux Elucidation in MFA Experiments," *Biotechnology & Bioengineering, Vol. 100*, No. 6, 1039-1049.
3. Satish Kumar V., M.S. Dasika, and C.D. Maranas (2007), "Optimization based automated curation of metabolic reconstructions," *BMC Bioinformatics, Vol. 8,* 212.
4. Suthers, P.F., A.P. Burgard, M.S. Dasika, F. Nowroozi, S. Van Dien, J.D. Keasling, and C.D. Maranas (2007), "Metabolic Flux Elucidation for Genome-Scale Models Using ^{13}C Labeled Isotopes," *Metabolic Engineering, Vol. 9*, 387-405.
5. Burgard, A., P. Pharkya and C.D. Maranas (2003), "OptKnock: A Bilevel Programming Framework for Identifying Gene Knock-Out Strategies for Microbial Strain Optimization," *Biotechnology & Bioengineering, Vol.84*, No. 6, 647-657.

(d) Synergistic Activities

1. Chair of Biochemical and Biomolecular Engineering XVII – *Emerging Frontiers* conference, Seattle WA, June 19-24, 2011.
2. Participant in Extreme Computing Opportunities in Biology workshop, Chicago IL, July 16, 2009.
3. Participant in the BER-NERSC Large Scale Computing and Storage Requirements for Biological and Environmental Research workshop, Rockville MD, May 7-8, 2009.
4. Fellow of the American Institute for Medical and Biological Engineering (Feb. 2008-present)
5. Editorial Board Member: PLoS Computational Biology, Biotechnology Journal, Biophysical Journal, and Metabolic Engineering

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors

Collaborators at Penn State include Profs. Reka Albert, Antonios Armaou, Stephen J. Benkovic, Howard Salis, Patrick Cirino, Michael Janik, Christine Keating, Gary Clawson, Thomas Wood, Greg Ferry and Tom Richard.

Collaborators and co-Editors outside Penn State include Prof. Maciek Antoniewicz (U. Delaware), Francois Banyex (U. of Washington), Beth Junger (Merck), Peter Karp (BioCyc), Anjum Muna (Veterinary Lab. Agency, UK), Himadri Pakrashi (Wash. U.), Lou Sherman (Purdue U.), Terry Papoutsakis (U. of Delaware), Mattheos Koffas (U. of Buffalo), James Liao (UCLA), Greg Stephanopoulos (MIT), Jay Keasling (UCB), Cathy H. Wu (U. of Delaware), Diego di Bernardo (U. of Naples), and Jason Papin (U. of Virginia).

ii. Graduate and Postdoctoral Advisors

Prof. Christodoulos A. Floudas, Princeton University (1990-1995)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor

Profs. K. Camarda (U. Kansas), Evgeni Nikolaev (Strayer Univ.) and Drs. G. Moore (Xencor, Inc.), S. Vaidyaraman (Eli Lilly), A. Burgard (Genomatica, Inc.), P. Pharkya (Genomatica, Inc.), A. Lehmann (Fox Chase Cancer Center), Mike Rodgers (DuPont), A. Gupta (SCA), M. Saraf (City Group), M. Dasika (Amyris), Hossein Fazelinia (Fox Chase Cancer Center), YoungJung Chang (CMU), Vinay Satish Kumar (JBEI), Francisco Vital-Lopez (BHSAI), and Sridhar Ranganathan (Life Technologies).

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NSF Engineering Research Center for Biorenewable Chemicals
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(a) Professional Preparation

Iowa State University	Economics	B.S., 1989
Iowa State University	Fisheries & Wildlife Biology	B.S., 1992

(b) Appointments

2008 – present	Assistant Director (Administrative Director), NSF Engineering Research Center for Biorenewable Chemicals, Iowa State University
2006 – 2008	Business Manager, Bioeconomy Institute, Iowa State University
2001 – 2006	Program Coordinator, Pre- and Post-Award Administration, Office of Biorenewables Programs, Iowa State University
1996 – 2001	Program Assistant, Center for Sustainable and Environmental Technologies, Iowa State University
1993 – 1996	Program Assistant, Center for Coal and the Environment, Iowa State University, and Fossil Energy Program, Ames Laboratory, US DOE

(c) Products

N/A

(d) Synergistic Activities

2010-2011, Chair, Equity, Diversity and Student-Athlete Well-Being Subcommittee, NCAA Certification Campaign, Iowa State University
2007-2011, Chair, Equity Committee, Athletics Council, Iowa State University
2005-2011, Member, Athletics Council, Iowa State University

(e) Collaborators and Other Affiliations

Collaborators and Co-Editors

N/A

Graduate Advisors and Postdoctoral Sponsors

N/A

Thesis Advisor and Postgraduate-Scholar Sponsor

N/A

MATTHEW NEUROCK
Alice M. & Guy A. Wilson Professor of Engineering, Chemical Engineering Department
University of Virginia
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(a) Professional Preparation

Michigan State University	Chemical Engineering	B.S., 1986
University of Delaware	Chemical Engineering	Ph.D., 1992
Schuit Institute of Catalysis	Chemical Engineering	Postdoc, 1992 - 1993

(b) Appointments

2005 – present	Alice M. & Guy A. Wilson Professor of Engineering, University of Virginia
2003 – present	Professor of Chemical Engineering, University of Virginia
2003 – present	Professor of Chemistry, University of Virginia
2000 – 2003	Associate Professor of Chemical Engineering, University of Virginia
2002	Technical Advisory Board for Heterogeneous Metathesis Catalysis, Dow Chemical Company
2001 – present	Editorial Board, Catalysis Communications
2001 – present	Board of Visitors, Department of Chemical Engineering, Michigan State Univ.
1995 – 1999	Assistant Professor of Chemical Engineering
1993 – 1995	*Visiting Research Scientist, DuPont Central Research and Development,* Corporate Catalysis Center, Experimental Station, Wilmington, DE.
1993 – 1995	Visiting Research Engineer, Department of Chemical Engineering, University of Delaware, Newark DE.

(c) Products

i. Five products most closely related to the proposed project

1. Hibbitts, D.D., and M. Neurock, A First Principles Analysis of the Influence of Oxygen and Alkalinity on the Selective oxidation of ethanol over Pd(111*), J. of Catal*., **299**, 261-271, 2013.
2. Sinha, N. and M. Neurock, A First Principles Analysis of the Hydrogenation of C_1-C_4 Aldehydes and Ketones over Ru(0001), *J. Catal.,* **295**, 31-44, 2012.
3. Ide, M., B. Hao, M. Neurock, and R.J. Davis, Mechanistic Insights on the Hydrogenation of a,b-unsaturated Ketones and Aldehydes to Unsaturated Alcohols over Metal Catalysts, *ACS Catal.,***2**, 4, 671-683, 2012
4. Chia, M., Y. Pagan-Torres, D. Hibbitts, Q.H. Tan, H.N. Pham, A. Datye, M. Neurock, R.J. Davis, and J.A. Dumesic, Selective Hydrogenolysis of Polyols and Cyclic Ethers over Bifunctional Surface Sites on Rhodium-Rhenium Catalysts, *J. Am. Chem. Soc.*, 133, 32, 12675-12689, 2011.
5. Zope, B.N., D.D. Hibbitts, M. Neurock, R.J. Davis, Reactivity of the Gold/Water Interface During Selective Oxidation Catalysis, *Science*, **330**, 6000, 74-78, 2010.

ii. Other significant products

1. Partha N., S. Hwang, W. Tang, M. Neurock, A. Katz, Catalytic Consequences of Open and Closed Grafted Al(III)-Calix[4]arene Complexes: Hydride and Oxo Transfer Reactions, Proc. Nat. Acad., **110**, 7, 2484-2489, 2013.
2. Zhu, Q., S.L. Wegener, C. Xie, O. Uuche, M. Neurock, and T. J. Marks, „Sulfur as a Soft Oxidation for the Catalytic Conversion of Methane", Nature Chemistry, **5**, 2, 104-109, 2013.

3. Sad, M.E., M. Neurock, and E. Iglesia, Formation of C-C and C-O Bonds and Oxygen Removal in Reactions of Alkanediols, Alkanols, and Alkanals on Copper Catalysts, J. Am. Chem. Soc., **133**, 50, 20384-20398, 2011.
4. Akpa, B.S., C.D'Agostino, L.F. Gladden, H. Manyar, K. Hindle, M. Neurock, D.W. Rooney, N. Sinha, E.H. Stitt, J.A. Zeitler and D. Weber, Solvent Effects in the Hydrogenation of 2-butanone, *J. Catal.*, **289**, 30-41, 2012.
5. Green, X., W. Tang, M. Neurock, and J.T. Yates, Jr., Spectroscopic Observation of Dual Catalytic Sites During Oxidation of CO on a Au/TiO_2 Catalyst, *Science*, **333**, 6043, 736-739, 2011.

(d) Synergistic Activities

Editor for the Journal of Catalysis
Panel Member: International Study by the World Technology Evaluation Center and the National Science Foundation, "Catalysis by Nanostructured Materials"
Advisory Board, Institute for Interfacial Catalysis, Pacific Northwest Lab., 2003- present
Director Catalysis and Reaction Engineering (Division 20), AICHE, (November 2003-2007)
Liaison of the South Eastern Catalysis Soc. to the North American Catalysis Soc. (2003-2006)

(e) Collaborators & Other Affiliations

i. Collaborators (last 48 months)

R. J. Davis, UVa
J. T. Yates, UVA
J. M. Dumesic, Wisconsin
G. Kraus, ISU
B. Shanks, ISU
A. Datye, UNM
E. Iglesia, UCB
A. Bell, UCB
M. Flytzani, Tufts
R. Gorte, Penn
G. Haller, Yale
H. Kung, NWU
M. Kung, NWU
T. Marks, NWU
J. Nørskov, Stanford
L. Pfefferle, Yale
W. Tysoe, UWM
S. Vajda, Argonne
R. van Santen, Eindhoven Univ. Tech.
I. Wachs, Lehigh
A.Wieckoski, UICU

ii. Graduate and Postdoctoral Advisors

Michael T. Klein (Rutgers Univ.), Rutger A. van Saten (Eindhoven Univ. of Tech., Netherlands)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (last 5 years)

Past Students:

Pallassana Venkataraman, Eric Hansen, Sanket Desai (Ph.D., Exxon Mobil), Hongmei Wen (Ph.D., United Tech.), Priyam Sheth (Ph.D., Shell Chemical Co.), Michael Janik (Ph.D., PSU), Christopher Taylor (Ph.D., LANL), Cheng Ying Lee, Neeti Kapur, Vamsi Vadhri, Nishant Sinha, David Hibbitts, Craig Plaisance. Postdocs: Dr. R. Meyer, Dr. Steven Mitchell, Dr. Sally Wasileski, Dr. Qingfeng Ge, Dr. Donghai Mei, Dr. Michael Palmer, Dr. Jean Sebastian Filhol, Dr. David Wathall, Dr. Yu Cai, Dr. Michael Janik, Dr. Christopher Taylor, Dr. Corneliu Buda, Dr. Jincheng Du, Dr. Lijun Xu, Dr. Wenjie Tang, Dr. Mohammed Haider, Dr. Tiana Raharintsalama

Current Students:

Bing Hao, Qiang Qian, Qiaohua Tan, Eric Dybeck, Erin Fitzharris, Fei Li, Tom Lawlor, Joe Marranca, Xuhui Feng.

BASIL J. NIKOLAU
Frances M. Craig Professor, Department of Biochemistry, Biophysics and Molecular Biology;
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(a) Professional Preparation

Massey University, New Zealand	Biochemistry/Chemistry	B.Sc., 1st Class Hon., 1977
Massey University, New Zealand	Biochemistry	Ph.D., 1982
University of California, Davis	Biochemistry	Post-doc - 1982-1983
University of Utah	Molecular Biology	Post-doc - 1983-1985

(b) Appointments

2008-present	Deputy Director, NSF Engineering Research Center for Biorenewable Chemicals (CBiRC)
2007-present	Director, Center of Metabolic Biology, ISU
2003-present	Director, W.M. Keck Metabolomics Research Laboratory, ISU
1999-2007	Director, Center for Designer Crops, ISU
1998-present	Professor, Department of Biochemistry, Biophysics and Molecular Biology, ISU
1993-1998	Associate Professor, Department of Biochemistry and Biophysics, ISU
1988-1993	Assistant Professor, Department of Biochemistry and Biophysics, ISU
1985-1988	Senior Scientist, Biotech. Department, Native Plants Inc., Salt Lake City, Utah

(c) Products

(i) *Five related*

1. Jin H, Nikolau BJ. 2012. The role of genetic redundancy in Polyhydroxyalkanoate Polymerases in PHA biosynthesis in *Rhodospirillum rubrum*. *Journal Bacteriology.* 194:5522-5529. PMID:22865850
2. Quanbeck SM, Brachova L, Campbell AA, Guan X, Perera A, He K, Rhee SY, Bais P, Dickerson JA, Dixon P, Wohlgemuth G, Fiehn O, Barkan L, Lange I, Lange BM, Lee I, Cortes D, Shuman J, Shulaev V, Huhman DV, Sumner LW, Roth MR, Welti R, Ilarslan H, Wurtele ES, **Nikolau BJ**. 2012. Metabolomics as a hypothesis-generating functional genomics tool for the annotation of *Arabidopsis thaliana* genes of "unknown function". *Frontiers in Technical Advances in Plant Science*. 2012;3:15. PMID:22645570
3. Lee YJ, Perdian DC, Song Z, Yeung ES, **Nikolau BJ**. 2012. Use of mass spectrometry for imaging metabolites in plants. *Plant Journal*. 70:81-95. PMID:22449044
4. Jin H, Song Z, **Nikolau BJ**. 2012. Reverse genetic characterization of two paralogous acetoacetyl CoA thiolase genes in Arabidopsis reveals their importance in plant growth and development. *Plant Journal*. 70:1015-32. PMID:22332816
5. Ding G, Che P, Ilarslan H, Wurtele ES, **Nikolau BJ**. 2012. Genetic dissection of methylcrotonyl CoA carboxylase indicates a complex role for mitochondrial leucine catabolism during seed development and germination. *Plant Journal*. 70:562-577. PMID:22211474

(ii) Five other significant

1. Li X, Ilarslan H, Brachova L, Qian HR, Li L, Che P, Wurtele ES, **Nikolau BJ**. 2011. Reverse-genetic analysis of the two biotin-containing subunit genes of the heteromeric acetyl-coenzyme A carboxylase in Arabidopsis indicates a unidirectional functional redundancy. *Plant Physiology* 155: 293-314. www.ncbi.nlm.nih.gov/pmc/articles/PMC3075786/
2. Jun JH, Song Z, Liu Z, **Nikolau BJ**, Yeung ES, Lee YJ. 2010. High-spatial and high-mass resolution imaging of surface metabolites of Arabidopsis thaliana by laser desorption-ionization mass spectrometry using colloidal silver. *Analytical Chemistry* 82: 3255-3265. www.ncbi.nlm.nih.gov/pubmed/20235569

3. Perera, MADN, Qin W, Yandeau-Nelson M, Fan L, Dixon P, **Nikolau BJ**. 2010. Biological origins of normal-chain hydrocarbons: a pathway-model based on cuticular wax analyses of maize silks. The Plant Journal 64: 618-632. www.ncbi.nlm.nih.gov/pubmed/21070415
4. Perera, MADN, Choi S-Y, Wurtele ES, **Nikolau BJ**. 2009. Quantitative analysis of short-chain acyl-Coenzyme-As in plant tissues by an LC-MS-MS electrospray ionization method. *Journal Chromatography B*, 877: 482-488. www.ncbi.nlm.nih.gov/pubmed/19157998
5. **Nikolau BJ**, Perera MA, Brachova L, Shanks B. 2008. Platform biochemicals for a biorenewable chemical industry. *Plant Journal*. 54: 536-545. www.ncbi.nlm.nih.gov/pubmed/18476861

(d) Synergistic Activities

1. Member of the scientific program organizing committee of the National Plant Lipid Cooperative (NPLC) (http://www.plantlipids.org/NPLC%202003Home.htm). Organized the 2001 and 2003 symposia, held biannually in June, at Lake Tahoe, CA.
2. Member of the International Advisory Board of the 2nd, 3rd, 4th and 5th International Congress on Plant Metabolomics, held 2003, 2004, 2006, and 2008.
3. Organizer of the 3rd International Conference on Plant Metabolomics, held in Ames, IA, June, 2004
4. Co-organizer of the 17th International Symposium on Plant Lipids, held 16-21 July 2006, at East Lansing, MI.
5. Editor of *Concepts in Plant Metabolomics* (2007). A 21-chapter book on plant metabolomics, Springer Press (ISBN-10: 1-4020-5607-9).

(e) Collaborators & Other Affiliations

- *Collaborators and Co-Editors*

Michael Beale (Rothamsted Research, England), Manfred Beckmann (Aberystwyth Univ), Raoul J. Bino (Wageningen Univ), Edgar Cahoon (U NE), Ping Che (U Western Australia), Elve Chen (U Hong Kong), Julie Dickerson (IA St U), Charles R. Dietrich (Monsanto), Philip Dixon (IA St U), Peter Dörmann (U Bonn), John Draper (Aberystwyth Univ), Beth Fatland (Archer Daniels Midland), Ivo Feussner (Univ of Goettingen), Oliver Fiehn (UC Davis), Margit Frentzen (Aachen Univ, Germany), Jennifer A. Gray (UC Davis), Allan Green (CSIRO Plant Industry, Australia), Ljerka Kunst (U British Columbia, Canada), Bernd Markus Lange (WA St U), David Meinke (OK St U), Stephanie Moon (Pioneer Hi-Bred), Rosanna Muralla (OK St U), Jonathan Napier, John B. Ohlrogge (MI St U), M.A.D.N. Perera (IA St U), Mike Pollard (MI St U), Seung Yon Rhee (Carnegie Institute), Owen Rowland (Carleton U, Canada), Kazuki Saito, Patrick S. Schnable (IA St U), Vladimir Shulaev (U North TX), Sten Stymne (Swedish U Agricultural Sciences), Lloyd W. Sumner (Samuel Roberts Noble Foundation), Lisa M. Weaver (Monsanto), Ruth Welti (KS St U), R Weselake (U Manitoba), Eve S. Wurtele (IA St U), Marna D. Yandeau-Nelson (IA St U), Hong Yao, Edward S. Yeung (IA St)

- *Graduate and Postdoctoral Advisors*

Roger Slack, retired; Clem Hawke, deceased; Paul K. Stumpf, deceased; Daniel F. Klessig, Boyce Thompson Institute for Plant Research

- *Thesis Advisor and Postgraduate Scholar Sponsor: 26 PhD; 14 MS; 12 Postdoctoral Fellows; 7 Research Associates*

PhD Students: *Past (16):* James Caffrey, Alexis Campbell, Ping Che, Joong-Kook Choi, Tomas Diez, Geng Ding, Joel Hansen, Huanan Jin, Yuqin Jin, Linsen Liu, Ann Perera, Joel Schmidt, Jianping Song, Li Xu, Xun Wang, Tuan-Nan Wen. *Current (10):* Daolin Cheng, Jennifer Chmielowski, Shivani Garg, Xin Guan, Fuyuan Jing, Adarsh Jose, Wenmin Qin, Kiran-Kumar Shivaiah, Lucas Showman, Bryon Upton. ***MS Students****: Past (12):* Elve Chen, Hsiaopo Cheng, Li Wei Cui, Jennifer Gray, Terence Hung-Wa Hui, Devlina Lahiri, Ping Li, Michael Tomas McDowell, Angela L. McKean, Stephanie Moon, Xiaobin Zheng. *Current (2):* Jason Hart, Jennifer Robinson. ***Postdoctoral Fellows:*** *Past (9):* Laura Civardi, Geng Ding, Beth Fatland, Vandana Mhaske, Cyril Periappuram, Cunxi Wang, Lisa M. Weaver, Lankun Wu, Wenxu Zhou. *Current (3):* Alexis Campbell, Huanan Jin, Zhihong Song. ***Scientists and Research Associates:*** *Past (3):* Wei Huang, Greg Keller, Stephanie Moon Quanbeck. *Present (4):* Libuse Brachova, Marna Yandeau-Nelson, Ludmila Rizshsky, Ann Perera

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Professor-Director & Investigator, Jack H. Skirball Center for Chemical Biology & Proteomics
Arthur and Julie Woodrow Chair, The Salk Institute for Biological Studies
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(a) Professional Preparation

University of Pittsburgh at Johnstown	Natural Sciences/Chemistry	B.S. – 1985
The Ohio State University	Chemistry/Biochemistry	Ph.D. – 1990
Yale University	Structural Biology	1990-1994

(b) Appointments

2005-present	Director, Jack H. Skirball Center for Chemical Biology and Proteomics, Salk Institute
2005-present	Professor, Jack H. Skirball Center for Chemical Biology and Proteomics, Salk Institute
2005-present	Investigator, Howard Hughes Medical Institute
2003-present	Adjunct Professor, Div. Biology and Dept. Chem. & Biochem., Univ. California, San Diego
2002-2004	Professor, Structural Biology Laboratory, Salk Institute
2000-2002	Adjunct Associate Professor, Depts. Biology and Chem. & Biochem., Univ. California, San Diego
1999-2002	Associate Professor, Structural Biology Laboratory, Salk Institute
1997-2000	Adjunct Assistant Professor, Dept. Biology, Univ. California, San Diego
1994-2000	Adjunct Assistant Professor, Dept. Chem. & Biochem., Univ. California, San Diego
1994-1999	Assistant Professor, Structural Biology Laboratory, Salk Institute
1990-1994	NSF Chemistry Postdoctoral Fellow, Professor Paul B. Sigler, Yale Univ.
1985-1990	Graduate Student, Dept. Chem., Ohio State Univ.

(c) Products (publications 136, citations 10,278, h-index = 50**)**
(http://scholar.google.com/citations?user=xiL1lscAAAAJ)
(i) Five Most Related to the Proposed Project

1. Zheng, Z.*, Guo, Y.*, Novák, O., Dai, X., Zhao, Y., Ljung, K., **Noel, J.P.***, and Chory J.* (2013) Coordination of auxin and ethylene biosynthesis by the aminotransferase VAS1. *Nat Chem Biol.* doi: 10.1038/nchembio.1178. Epub ahead of print 2013 Feb 3. PubMed PMID: 23377040. (*equal contributions)
2. Weng, J.-K. and **Noel, J.P.** (2012) Structure-Function Analyses of Plant Type III Polyketide Synthases. *Methods Enzymol.* **515**: 317-335. PubMed PMID: 22999180.
3. Weng, J.-K., Philippe, R.N. and **Noel, J.P.** (2012) The rise of chemodiversity in plants. *Science* **336**: 1667-1670. PubMed PMID: 22745420.
4. Ngaki, M.N.*, Louie, G.V.*, Philippe, R.*, Manning, G., Pojer, F., Bowman, M.E., Ling, Li, Larsen, E., Syrkin Wurtele, E. and **Noel, J.P.** (2012) Evolution of the Chalcone-Isomerase Fold from Fatty-Acid Binding to Stereospecific Catalysis. *Nature* **485**: 530-533. doi:10.1038/nature11009. PubMed PMID: 22622584. (*equal contributions).
5. Auldridge, M.E., Austin, M.B., Ramsey, J., Fridman, E., Pichersky, E. and **Noel, J.P.** (2012) Emergent decarboxylase activity and attenuation of hydrolase activity during the evolution of

methylketone biosynthesis in tomato. *Plant Cell* **24**: 1596-1607. Epub 2012 Apr 20. PubMed PMID: 22523203; PubMed Central PMCID: PMC3398566.

(ii) Five Other Significant Products

1. Weng, J.K. and **Noel, J.P.** (2012) The Remarkable Pliability and Promiscuity of Specialized Metabolism. *Cold Spring Harb Symp Quant Biol.* Epub ahead of print 2012 Dec 26. PubMed PMID: 23269558.
2. **O'Maille, P.E.**, **Malone, A.**, **Dellas, N.**, Hess, B.A., Smentek, L., **Sheehan, I.**, Greenhagen, B.T., Chappell, J., Manning, G. and **Noel, J.P.** (2008) Quantitative exploration of the catalytic landscape separating divergent plant sesquiterpene synthases. *Nat. Chem. Biol.* **4**: 617-623. Epub 2008 Sep 7. PubMed PMID: 18776889; PubMed Central PMCID: PMC2664519.
3. Louie, G.V., Baiga, T.J., Bowman, M.E., Koeduka, T., Taylor, J.H., Spassova, S.M., Pichersky, E. and Noel, J.P. (2007) Structure and reaction mechanism of basil eugenol synthase. *PLoS ONE.* 10: e993. PubMed PMID: 17912370; PubMed Central PMCID: PMC1991597.
4. Yu, G.*, Nguyen, T.T.*, **Guo, Y.***, Schauvinhold, I., **Auldridge, M.E.**, Bhuiyan, N., Ben-Israel, I., Iijima, Y., Fridman, E., **Noel, J.P.** and Pichersky, E. (2010) Enzymatic functions of wild tomato methylketone synthases 1 and 2. *Plant Physiol.* **154**: 67-77. Epub 2010 Jul 6. PubMed PMID: 20605911; PubMed Central PMCID: PMC2938155 (*equal contributions).
5. **Kuzuyama, T.**, **Noel, J.P.**, and **Richard, S.B.** (2005) Structural basis for the promiscuous biosynthetic prenylation of aromatic natural products. *Nature* **435**: 983-987. PubMed PMID: 15959519; PubMed Central PMCID: PMC2874460.

(d) Synergistic Activities

Panel Member-National Science Foundation; Medical Student Course (UCSD) Herbal Remedies, Functional Foods, and Natural Products (11th Yr); Mentor for Salk Life Science Summer Institute program; Public Outreach Seminars through Salk's Taste of Discovery Series; Member, Science & Impact Advisory Board, John Innes Centre; Member, Scientific Advisory Board, Donald Danforth Plant Science Center; Member, Scientific Advisory Board, Max Planck Research Unit for Enzymology of Protein Folding; Member, External Advisory Committee for Pharmacology, Vanderbilt University; Editorial Advisory Board, ACS Chemical Biology; Associate Editor, The Plant Cell; Co-Founder & Chair Scientific Advisory Board, Allylix, Inc., Co-Founder, Pareto Biotechnologies, Inc., AAAS Fellow

(e) Collaborators & Other Affiliations

(i) Collaborators - Michael Burkart (UCSD), Joseph Chappell (University of Kentucky), Clint Chapple (Purdue), Richard Dixon (Noble Foundation), Natalia Dudareva (Purdue), Lutz Heide (Univ. Tubingen), Rob Kay (MRC-Cambridge), Tomohisa Kuzuyama (Univ. Tokyo), Bradley Moore (UCSD), Eran Pichersky (Univ. Michigan), Joseph Schroeder (Univ. Freiburg), Vladimir Shulaev (Virginia Tech), Brenda Winkel-Shirley (Virginia Tech)

(ii) Graduate and Postdoctoral Advisors - Ming-Daw Tsai (The Ohio State University, Ph.D. Training), Paul B. Sigler – deceased (Yale University, Postdoctoral Training)

(iii) Undergraduate Mentor, Thesis Advisor and Postgraduate-Scholar Sponsor - Erin Bomati (Illumina), Jean-Luc Ferrer (ESRF), Joseph M. Jez (Danforth), Courtney Starks (Sequoia), Chloe Zubieta (Stanford), Mark Verdecia (NIH), Paul O'Maille (John Innes), Rama Ranganathan (UT-Southwestern), Ryan Phillipe (Manus), Chang-Jun Liu (Brookhaven), Yan (Jessie) Zhang (UT-Austin), Alexandrine Bilwes (Cornell), Toyoyuki Ose (Hokkaido University), David Liscombe (Vineland Research & Innovation Centre)

Total Number of Postdoctoral Researches (current and past) – 33
Total Number of Graduate Students-MS and PhD (current and past) – 10
Total Number of Undergraduate Students (current and past) – 31
Total Number of High School Students (current and past) – 5

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(a) Professional Preparation

Iowa State University	Chemical Engineering	BS 2002
University of Iowa	Chemical and Biochemical Engr.	PhD 2008
University of Iowa	Chemical and Biochemical Engr.	Postdoc 2008

(b) Appointments *(list in reverse chronological order beginning with the most current appointment)*

2011 – 2012	Translational Research Manager; NSF Engineering Research Center for Biorenewable Chemicals, Iowa State University
2009 – 2011	Biochemical Engineer/Sr. Research Scientist, Bioprocessing; Novus International, Inc.
2008 – 2009	Scientist; Optherion, Inc.
2008 – 2008	Postdoctoral Researcher; University of Iowa
2006 – 2006	Intern, Biochemistry; Genencor International
2002 – 2008	Graduate Student Research Assistant; University of Iowa

(c) Products

i. Five products most closely related to the proposed project

none

ii. Five other significant products

Okerlund, A. L. 2008. *Naphthalene dioxygenase structure-function analysis*, PhD thesis, The University of Iowa.

Liang, H., Whited, G., Nguyen, C., Okerlund, A. & Stucky, G. D. 2008. Inherently tunable electrostatic assembly of membrane proteins. *Nano Letters,* 8**,** 333-339.

Ferraro, D. J., Okerlund, A. L., Mowers, J. C. & Ramaswamy, S. 2006. Structural basis for regioselectivity and stereoselectivity of product formation by naphthalene 1,2-dioxygenase. *Journal of Bacteriology,* 188**,** 6986-6994.

(d) Synergistic Activities (*list up to 5 examples that demonstrate the broader impact of your professional/scholarly activities that focus on the integration/transfer of knowledge as well as its creation*)

NSF Engineering Research Center for Biorenewable Chemicals – At the NSF Engineering Research Center for Biorenewable Chemicals (CBiRC) I am coordinating efforts to advance academic research into viable scalable processes.

Novus International, Inc. - At Novus International I developed several processes for metabolite purification from a complex fermentation broth. After feasibility tests and cost reduction steps were implemented, I designed and developed a scale-up procedure to increase purification levels from milligram quantities of the metabolite to kilogram quantities.

(e) Collaborators & Other Affiliations *(please list alphabetically in each section)*

i. Collaborators and Co-Editors (past 48 months)

Name	Organizational Affiliation
none	

ii. Graduate Advisors and Postdoctoral Sponsors (your own)

Name	Organizational Affiliation
none	

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Name	Organizational Affiliation
none	

0 = Total number of graduate students advised and postdocs sponsored

ERAN PICHERSKY
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---

(a) Professional Preparation

University of California, Berkeley	Genetics	B.Sc. 1980
University of California, Davis	Genetics	Ph.D. 1984
Rockefeller University	Molecular Biology	Post-doc, 1984-1987

(b) Appointments

2009	Visiting Professor, Australian National University, Department of Botany and Zoology, Canberra, Australia
2001-2003	Interim Chair and Chair, Department of Molecular, Cellular, and Developmental Biology (MCDB), University of Michigan
2001-present	Professor, MCDB Department, University of Michigan
2001	Visiting Professor, Hebrew University of Jerusalem, Faculty of Agriculture
2000	Visiting Alexander von Humboldt Forschungspreistrager and Senior Fulbright Scholar, Max-Planck-Institute for Chemical Ecology, Jena, Germany
1998 – 2001	Professor, Department of Biology, University of Michigan
1995 - 2000	Associate Chair for Research and Facilities, Biology Department, University of Michigan
1993	visiting Associate Professor, Institute of Biological Chemistry, Washington State University, Pullman, WA
1992 - 1998	Associate Professor, Department of Biology, University of Michigan
1986 - 1992	Assistant Professor, Department of Biology, University of Michigan

(c) Products

i. Five products most closely related to the proposed project

1. Falara V, Amarasinghe R, Poldy J, Pichersky E, Barrow R, Peakall R. The production of a key floral volatile is dependent on UV light in a sexually deceptive orchid. Ann Bot, in press.
2. Park J, María Rodríguez-Moyá, Li M, Pichersky E, San K-Y, Gonzalez. Synthesis of methyl ketones by metabolically engineered *Escherichia coli.* Journal of Industrial Microbiology & Biotechnology 39:1703-1712 (2012).
3. Yu G, TTH Nguyen, Y Guo, I Schauvinhold, ME Auldridge, N Bhuiyan, E Fridman, Y Iijima, JP Noel, E Pichersky. The enzymatic functions of the wild tomato *Solanum habrochaites glabratum* methylketone synthases 1 and 2. Plant Physiol 154:67-77 (2010).
4. Ben-Israel I, G Yu, MB Austin, N Bhuiyan, M Auldridge, T Nguyen, I Schauvinhold, JP Noel, E Pichersky, E Fridman. Multiple biochemical and morphological factors underlie the production of methylketones in tomato trichomes. Plant Physiology 151:1952-1964 (2009).
5. Fridman E, Wang J, Iijima Y, JE Froehlich, Gang DR, Ohlrogge J, E Pichersky. Metabolic, genomic, and biochemical analyses of glandular trichomes from the wild tomato species *Lycopersicon hirsutum* identify a key enzyme in the biosynthesis of methylketones. Plant Cell 17:1252-1267 (2005).

ii. Five other significant products

1. Schilmiller AL, I Schauvinhold, M Larson, R Xu, AL Charbonneau, A Schmidt, C Wilkerson, RA Last, E Pichersky. Monoterpenes in the glandular trichomes of tomato are synthesized via a neryl diphosphate intermediate rather than geranyl diphosphate. Proc Natl Acad Sci USA, 106:10865-10870 (2009).

2. Schilmiller AL, RL Last, E Pichersky. Harnessing plant trichome biochemistry for the production of useful compounds. Plant J 54:702-711 (2008).
3. Davidovich-Rikanati, R, Y Sitrit, Y Tadmor, Y Iijima, N Bilenko, E Bar, B Carmona, N Dudai, JE Simon, E Pichersky, E Lewinsohn. Enrichment of tomato flavor by diversion of the early plastidial terpenoid pathway. Nature Biotech. 25:899-901 (2007).
4. Koeduka T, E Fridman, DR Gang, DG Vassão, BL Jackson, CM Kish, I Orlova, SM Spassova, NG Lewis, JP Noel, TJ Baiga, N Dudareva, E Pichersky. Eugenol and isoeugenol, charecteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. Proc Natl Acad Sci (USA) 103:10128-10133 (2006).
5. Pichersky E, JP Noel, N Dudareva. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. Science 311:808-811 (2006).

(d) Synergistic Activities

Developed and taught for 10 years a "project lab" in plant molecular biology and biochemistry for undergraduates.

Interviewed by National Public Radio and numerous other voice and print media outlets concerning work on plant aroma biology done in my lab, with many news articles published (most recently, in the April 13, 2010 issue of the Washington Post)

Work from my lab, including an interview with me, was featured on PBS's science program, "Secrets of the Sequence".

Published an article in the lay science magazine *American Scientist* on plant volatiles.

Chaired a Gordon Conference on Plant Volatiles in 2007.

Served as Plant Biochemistry Panel Manager, AFRI, USDA, 2009.

(e) Collaborators & Other Affiliations

i. Collaborators (past 48 months)

Cornelius Barry (Michigan State University)
Gilles Basset (University of Nebraska)
Dr. Natalia Dudareva (Purdue University)
Robert Last (Michigan State Univ)
Efraim Lewinsohn (ARO – Israel)
Joseph Noel (Salk Institute)
Rod Peakall (Australian National University, Australia)
Alexander Vainstein (Hebrew University, Israel)

ii. Graduate Advisors and Postdoctoral Sponsors

Dr. Leslie Gottlieb, UC Davis (deceased)
Post-doc Advisor: Dr. Anthony Cashmore, U. Penn (retired)
Sabbaticals with:
Dr. Rodney Croteau (Washington State University, 1993),
Dr. Jonathan Gershenzon (Max Planck Institute, Jena, 2000)
Dr. Efraim Lewinsohn (Newe Ya'ar Research center, Israel, 2001)
Rod Peakall (Australian National University, Australia, 2009)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years):

Graduate students:	*Post-docs*:	Yuki Matsuba
Adam Schmidt	Tariq Akhtar	Thuong Nguyen
Yue Yang	Nazmul Bhuiyan	Susanna Roeder
Geng Yu	Vasiliki Falara	Ines Schauvinhold
	Eyal Fridman	Goro Taguchi
	Mwafaq Ibdah	Marina Varbanova
	Yoko Iijima	Jihong Wang
	Takao Koeduka	Guodong Wang

Total number of graduate students: >10 Total number of post-docs: >25

D. RAJ RAMAN, PHD, PE
Professor and Associate Chair for Teaching, Agricultural and Biosystems Engineering
Iowa State University
3222 NSRIC, Ames, IA 50011-3310
(515) 294-0465 / (515) 294-4250 (fax) / rajraman@iastate.edu

(a) Professional Preparation

Rochester Institute of Technology	Electrical Engineering	B.S., 1986
Cornell University	Ag. & Biological Engineering	Ph.D., 1994

(b) Appointments

2011 - present	Associate Head for Teaching, ISU[1] ABE[2]
2010 - present	Pyrone Testbed Chamption, CBiRC[3]
2008 – present	University Education Program Director, CBiRC
2006 – present	Assoc. Prof./Professor Agricultural & Biosystems Engineering, ISU
2006 – 2010	Assoc. Director of Educational Programs, ISU Bioeconomy Institute
2004 – 2005	Interim Head, Biosystems Engineering and Soil Science, UTK[4]
1999 – 2005	Assoc. Professor, Biosystems Engineering, UTK
1993 – 1999	Asst. Professor, Biosystems Engineering, UTK

(c) Products

1. Christiansen, K. L., D. R. Raman, and R. P. Anex. 2012/13. Predicting cost growth and performance of first-generation algal production systems. *Energy Policy* 51: 382 - 391
2. Faulhaber, C. R., D. R. Raman, and R. T. Burns. 2012. An engineering-economic model for analyzing dairy plug-flow anaerobic digesters: cost structures and policy implications. *Transactions of the American Society of Agricultural and Biological Engineers* 55(1): 201 – 209
3. Raman, D. R. and R. P. Anex. 2012. Conceptual and mathematical models of batch simultaneous saccharification and fermentation: dimensionless groups for predicting process dynamics. *Journal of Biological Systems* 20(2): 195 – 211
4. Rawat, V., D. R. Raman, and R. P. Anex. 2011. Technical Note: Detecting and Subcategorizing Hard-Coding Errors in Bioenergy-Relevant Spreadsheets using Visual Basic for Applications (VBA). *Applied Engineering in Agriculture* 27(3): 469-474
5. Cruse, M. J., M. Z. Liebman, D. R. Raman, M. H. Wiedenhoeft. 2010. Fossil Energy Use in Conventional and Low-External-Input Cropping Systems. *Agronomy Journal* 102(3): 934-941

Selected Recent Products – 5 Other

6. Kaleita, A. L., and D. R. Raman. 2012/13. A rose by any other name: an analysis of agricultural and biological engineering undergraduate curricula. *Transactions of the ASABE* 55(6): 2371-2378
7. Haen, K. M., D. R. Raman, E. Polush, and M. R. Kemis. 2012. Training the Next Generation of Creative, Innovative and Adaptive Scientists and Engineers: The NSF Engineering Research Center for Biorenewable Chemicals (CBiRC) Research Experience for Undergraduates. *Education for Chemical Engineers* (*Accepted*, available online @ http://dx.doi.org/10.1016/j.ece.2012.09.001)

[1] Iowa State University
[2] Agricultural and Biosystems Engineering
[3] NSF Engineering Research Center for Biorenewable Chemicals
[4] The University of Tennessee, Knoxville

8. Murphy, P. T., K. J. Moore, D. R. Raman, R.P. Anex, and S. L. Fales. 2012. Rapid biomass quality determination of corn stover using near infrared reflectance spectroscopy. *BioEnergy Research*. 5(1): 79 – 85
9. Himmelsbach, J. N., D. R. Raman, R. P. Anex, R. T. Burns, and C. R. Faulhaber. 2010. Effect of ammonia soaking pretreatment and enzyme addition on biochemical methane potential of switchgrass. *Transactions of the ASABE.* 53(6): 1921-1927
10. Haney, L. J., J. G. Coors, A. J. Lorenz, D. R. Raman, R. P. Anex, and M. P. Scott. 2008. Development of a fluorescence-based method for monitoring glucose catabolism and its potential use in a biomass hydrolysis assay. *Biotechnology for Biofuels* 2008, **1**:17

(d) Synergistic Activities

Testbed Champion (providing technoeconomic and lifecycle assessments for *pyrone testbed*) – NSF ERC for Biorenewable Chemicals (CBiRC)

University Education Program Director – CBiRC

Objective 8 (Education) Co-Director, CenUSA Sustainable Production and Distribution of Bioenergy for the Central USA (USDA AFRI- CAP Program)

Engineering Curriculum Committee Chair, Agricultural and Biosystems Engineering Department (overseeing curricula and ABET accreditation of the Agricultural Engineering and the Biological Systems Engineering degree programs)

(e) Collaborators & Other Affiliations

Collaborators (past 48 months)

Robert Anex (ISU), Robert Brown (ISU), Thomas Brumm (ISU), John Buchanan (Univ. of Tennessee), Robert Burns (ISU), Jim Coors (Univ. of Wisconsin), Czar Crofcheck (Univ. of Kentucky), Jill Euken (ISU), Reid Gerhardt (Univ. of Tennessee), Lisa Haney (Syngenta), Brian He (Univ. of Idaho), Larry Johnson (ISU), Alice Layton (Univ. of Tennessee), Jaehoon Lee (Univ. of Tennessee), Ken Moore (ISU), Michael Mullen (Univ. of Kentucky), Sue Nokes (Univ. of Kentucky), Anthony Pometto (ISU), Steven Ricke (Univ. of Arkansas), Bruce Robinson (Univ. of Tennessee, Ret.), Gary Sayler (Univ. of Tennessee), Marvin Scott (ISU/USDA ARS), Jon VanGerpen (Univ. of Idaho), David White (Univ. of Tennessee), John Wilkerson (Univ. of Tennessee), Elizabeth Williams (Central Carolina Comm. College), James Wills (Univ. of TN)

Graduate Advisor: Larry P. Walker (Cornell University)

Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

Past Students (48 mo): Patrick Murphy (PhD), Jasjeet Kaur (MS), Jenni Himmelsbach (MS), Carol Faulhaber (MS), Vertika Rawat (MS), Katrina Christiansen (PhD), Darren Jarboe (PhD; no relation to Laura Jarboe)

Current Students: Joshua Claypool (MS expected 2013).

PETER J. REILLY
Professor of Chemical and Biological Engineering and Anson Marston Distinguished Professor in Engineering, Department of Chemical and Biological Engineering
Iowa State University
Ames, IA, 50011-2230
515-294-5968 / 515-294-2689 (fax) / E-mail address: reilly@iastate.edu

(a) Professional Preparation

Princeton University	Chemistry	A.B., 1960
University of Pennsylvania	Chemical Engineering	Ph.D., 1964

(b) Appointments

2005 – Present	Professor of Chemical and Biological Engineering, ISU
1992 – Present	Anson Marston Distinguished Professor in Engineering, ISU
1979 – 2005	Professor of Chemical Engineering, ISU
1974 – 1979	Associate Professor of Chemical Engineering, ISU
1968 – 1974	Assistant Professor of Chemical Engineering, Univ. of Nebraska
1964 – 1968	Research Engineer, E. I. du Pont de Nemours & Company, Deepwater, NJ

(c) Products

i. Five products most closely related to the proposed budget

1. Johnson, G. P., L. Petersen, A. D. French, and P. J. Reilly. Twisting of Glycosidic Bonds by Hydrolases. *Carbohydr. Res*., **344**, 2157 (2009).
2. Warner, C. D., J. A. Hoy, T. C. Shilling, M. J. Linnen, N. D. Ginder, C. F. Ford, R. B. Honzatko, and P. 2. J. Reilly. Tertiary Structure and Characterization of a Glycoside Hydrolase Family 44 Endoglucanase from *Clostridium acetobutylicum*. *Appl. Environ. Microbiol.*, **76**, 338 (2010).
3. Petersen, L., A. Ardèvol, C. Rovira, and P. J. Reilly. Molecular Mechanism of the Glycosylation Step Catalyzed by Golgi a-Mannosidase II. A QM/MM Metadynamics Investigation. *J. Am. Chem. Soc.,* **132**, 8291 (2010).
4. Cantu, D. C., Y. Chen, and P. J. Reilly. Thioesterases: A New Perspective Based on Their Primary and Tertiary Structures. *Protein Sci.,* **19**, 1281 (2010).
5. Barker, I. J., L. Petersen, and P. J. Reilly. Mechanism of Xylobiose Hydrolysis by GH43 b-Xylosidase. *J. Phys. Chem. B,* **114**, 15389 (2010).

ii. Five other significant products

1. Warner, C. D., R. M. Go, C. García-Salinas, C. Ford, and P. J. Reilly. Kinetic Characterization of a Glycoside Hydrolase Family 44 Endoglucanase from *Ruminococcus flavefaciens* FD-1. *Enzyme Microb. Technol*., **48**, 27 (2011).
2. Cantu, D. C., Y. Chen, M. L. Lemons, and P. J. Reilly. ThYme: A Database for Thioester-Active Enzymes. *Nucleic Acids Res.,* **39**, D342 (2011).
3. Jing, F., D C. Cantu, J. Tvaruzkova, J. P. Chipman, B. J. Nikolau, M. D. Yandeau-Nelson, and P. J. Reilly. Phylogenetic and Experimental Characterization of an Acyl-ACP Thioesterase Family Reveals Significant Diversity in Enzymatic Specificity and Activity. *BMC Biochem.*, **12**, 44 (2011).
4. Chen, Y., E. E. Kelly, R. P. Masluk, C. L. Nelson, D. C. Cantu, and P. J. Reilly. Structural Classification and Properties of Ketoacyl Synthases. *Protein Sci.*, **20**, 1659 (2011).

5. Hill, A. D., and P. J. Reilly. Scoring Functions for AutoDock. *Molecular Methods in Biochemistry*. M. Frank and T. Lüttke, eds. Part III – Structural Bioinformatics. Springer. Accepted for publication.

(d) Synergistic Activities

Speakers' Bureau member, American Chemical Society, 1984–2009 (68 sections visited)
Speakers' Bureau member, American Institute of Chemical Engineers, 1987–2000 (27 sections visited)
Advisor, Iowa State University Chapter, Society of Hispanic Professional Engineers, 1986–1992
Coordinator, Iowa State University–University of Glasgow Exchange, 1984–2002; Iowa State University–Université de Lausanne–Ecole Polytechnique Fédérale de Lausanne Exchange, 1985–present
Since 1999, my refereed publicatons have had 22 different undergraduate coauthors; nine of those papers have had undergraduate first authors.

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Ardevol, Albert	University of Barcelona, Spain
Ford, Clark	Iowa State University
French, Alfred	USDA Southern Regional Research Center
Fushinobu, Shinya	University of Tokyo, Japan
Ginder, Nathaniel	Washington University Medical School
Gu, Xun	Iowa State University
Hidaka, Masafumi	National Food Research Institute, Japan
Honzatko, Richard	Iowa State University
Hoy, Julie	University of Guelph, Canada
Johnson, Glenn	University of Iowa
Kitaoka, Motomitsu	National Food Research Institute, Japan
Lemons, Matthew	Iowa State University
Nerinckx, Wim	Ghent University, Belgium
Nikolau, Basil	Iowa State University
Rovira, Carme	University of Barcelona, Spain
Yandeau-Nelson, Marna	Iowa State University

ii. Graduate and Postdoctoral Advisors (your own)

Arthur E. Humphrey	Retired

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

Cantu, David	Iowa State University
Chen, Yingfei	Iowa State University
Hill, Anthony	St. Jude Medical Center, Minneapolis
Mertz, Blake	University of Arizona, Tucson, Ariz.
Peterson, Luis	OPKO Pharmaceutical Co., Guadalajara, Mexico
Shilling, Taran	Houston, Tex.
Vander Velden, Kent	Pioneer Hi-Bred Seeds, Johnston, Ia.
Warner, Christopher	Scripps Research Institute, Jupiter, Fla.

57 = Total number of graduate students advised and postdocs sponsored.

KA-YIU SAN
Professor, Department of Bioengineering
Rice Unviersity
6100 Main Street, MS 142
Houston, Texas 77005
713-348-5361/713-348-5877 (fax) / ksan@rice.edu

(a) Professional Preparation

Rice University	Chemical Engineering	B.S., 1978
California Institute of Technology	Chemical Engineering	M.S., 1981
California Institute of Technology	Chemical Engineering	Ph.D., 1984
California Institute of Technology	Biochemical Engineering	Jan. 1984 – July 1984

(b) Appointments

2004-present	E.D. Butch Professor in Bioengineering, Rice University
1996-2004	Professor, Bioengineering, Rice University
1996-present	Professor, Chemical and Biomolecular Engineering, Rice University
1990-1996	Associate Professor, Chemical Engineering, Rice University
1984-1990	Assistant Professor, Chemical Engineering, Rice University

(c) Products

i. Five products most closely related to the proposed project

1. Martínez, I., Zhu, J., Lin, H., Bennett, G.N., San, K.-Y., " Replacing Escherichia coli NAD-dependent glyceraldehyde 3-phosphate dehydrogenase (GAPDH) with a NADP-dependent enzyme from *Clostridium acetobutylicum* facilitates NADPH dependent pathways, Metabolic Engineering, 10(6):352-359 (2008).
2. Sánchez, A. M., Bennett, G. N., and San, K.-Y., Novel pathway engineering design of the anaerobic central metabolic pathway in *Escherichia coli* to increase succinate yield and productivity, Metabolic Engineering, 7(3):229-239 (2005).
3. Zhang X, Li M, Argawal A, San K-Y. Efficient free fatty acid production in *Escherichia coli* using plant acyl-ACP thioesterases. Metabolic Engineering, 13:713-722 (2011).
4. Zhang X, Argawal A, San K-Y. Improving fatty acid production in *Escherichia coli* through the overexpression of malonyl coA-Acyl carrier protein transacylase. Biotechnology Progress, 28:60-65 (2012).
5. Li M, Zhang X, Argawal A, San K-Y. Effect of acetate formation pathway and long chain fatty acid CoA-ligase on the free fatty acid production in *E. coli* expressing acyl-ACP thioesterase from *Ricinus communis*. Metabolic Engineering, 14: 380-387 (2012).

ii. Five other significant products

1. Zhu, J., Shalel Levanon, S., Bennett, G. N., and San, K.-Y., The YfiD protein contributes to the pyruvate formate-lyase flux in an *E. coli* arcA mutant strain, Biotechnology and Bioengineering, 97:138-43 (2007).
2. Shalel-Levanon, S., San, K.-Y., and Bennett, G. N., Effect of oxygen on the *E. coli* ArcA and FNR regulation systems and metabolic responses, Biotechnology and Bioengineering, 89:556-64 (2005).
3. Sánchez, A. M., Bennett, G. N., and San, K.-Y., Batch culture characterization and metabolic flux analysis of succinate producing *E. coli* strains, Metabolic Engineering, 8:209–226 (2006).
4. Peebles CAM, Sander GW, Hughes EH, Peacock R, Shanks JV, San K-Y. The expression of 1-deoxy-D-xylulose synthase and geraniol 10-hydroxylase or anthranilate synthase increases terpenoid indole alkaloid accumulation in *Catharanthus roseus* hairy roots. Metabolic Engineering, 13:234-40 (2011).
5. Zhu J, Sanchez A, San K-Y, Bennett GN. Manipulating Respiratory Levels in *Escherichia coli* for Aerobic Formation of Reduced Chemical Products. Metabolic Engineering, 13:704-712 (2011).

(d) Synergistic Activities

Co-author of a textbook, "Bioengineering Fundamentals", with Ann Saterbak and Larry V. McIntire (Prentice Hall, January 2007). The textbook is intended for sophomore level bioengineering introductory course. The book emphasizes on the quantitative treatment of conservation principles using biological systems as examples.

Involved in teaching a course in biochemical engineering;

Many strains of bacteria and plasmids constructed in the lab have been sent to colleagues throughout the world.

Currently serve on the editorial board of journals in the area of biochemical and metabolic engineering.

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

George N. Bennett	Rice University
Ateeque Ahmad	Konkuk University, S. Korea
Ill-Min Chung	Konkuk University, S. Korea
Sue Gibson	University of Minnesota
Ramon Gonzalez	Rice University
Kathleen Mathews	Rice University
Jackie V. Shanks	Iowa State
M. Karanjikar	Tech Holding
PraveenV. Vadlani	Kansas State University
Kris Prather	MIT
Chandresh Thakker	Rice University
Ching C. Lau	Baylor College of Medicine

ii. Graduate Advisors and Postdoctoral Sponsors

Gregory N. Stephanopoulos	California Institute of Technology (Graduate advisor)
Gregory N. Stephanopoulos	California Institute of Technology (Postdoctoral advisor)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)*

Stephanie Porter	Glycos Biotechnologies
Christie Peebles	Colorado State University
Irene Martinez	Pontifical Catholic University of Valparaiso, Chile
Joanna Jan	Rice
SongI Han	Current, Rice
Jiangfeng Zhu*	Tech Univ of Denmark
Mathew Wong*	Glycos Biotechnologies
Grant Blazer*	NREL
Mai Li*	Glycos Biotechnologies
Yane Luo*	Northwest University, China
Haijun Gao*	Beijing Institute of Technology, China
Leepika Tuli*	Current, Rice
Xixian X*	Current, Rice
Hui Wu*	Current, Rice
Wei Li*	Current, Rice
Xuijun Zhang*	Rice

16 = graduate students advised and postdocs/visiting scientists sponsored.

SUZANNE B. SANDMEYER
Professor, Biological Chemistry and Microbiology & Molecular Genetics
Associate Director, UCI Institute for Genomics and Bioinformatics
Director, Protein and DNA Mircoarray Facility
University of California, Irvine
Dept. of Biological Chemistry, D240 Med Sci I, Irvine, CA 92697-1700
(949) 824-7571/(949) 824-2688 (fax)/sbsandme@uci.edu

(a) Professional Preparation

Carleton College	Biology	B.A., 1973
University of Washington	Biochemistry	Ph.D., 1980
Washington University	Genetics	Postdoc, 1980 – 1982

(b) Appointments

2011-present	Professor, Chemical Engineering and Materials Science, School of Engineering
2009 – present	Associate Director, UCI Institute for Genomics & Bioinformatics, University of California, Irvine (UC-Irvine)
2000 – present	Director, Genomics High-Throughput Facility, UC-Irvine
1997 – present	Professor, Biological Chemistry, UC-Irvine (primary appointment)
1997 – 2005	Chair, Dept. of Biological Chemistry, UC-Irvine
1994 – present	Professor, Microbiology & Molecular Genetics, UC-Irvine
1990 – 1994	Assoc. Professor, Microbiology & Molecular Genetics, UC-Irvine
1984 – 1990	Asst. Professor, Microbiology & Molecular Genetics, UC-Irvine
1982 – 1983	Research Associate, Genetics, Washington University, St. Louis, MO
1974 – 1980	Research Associate, Biochemistry University of Washington, Seattle, WA
1973 – 1974	Teaching Assistant, Biochemistry, University of Washington, Seattle, WA

(c) Products

i. Five products most closely related to the proposed project (yeast vectors, bioengineering, and retrotrotransposon strain modifications)

1. Qi, X., Daily, K., Nguyen, K., Wang, H., Mayhew, D., Rigor, P., Forouzan,S., Johnston, M., Mitra, R.D., Baldi, P. and **Sandmeyer, S.B.** (2012) Retrotransposon profiling of RNA polymerase III initiation sties Retrotransposon profiling of RNA polymerase III initiation sites. Genome Res. Online Jan 27, 2012, doi:10.1101/gr.131219.111.
2. Fang, F., Salmon, K., Shen, M., Aeling, K, Ito, E., Irwin, B., Tran, U., Hatfield, G.W., Da Silva, N.*, and Sandmeyer, S.* (2011) A combinatorial vector set for metabolic engineering in *Saccharomyces cerevisiae*. Yeast 28:123-136.
3. Clemens, K., Larsen, L.Z., Zhang, M., Kuznetsov, Y., Bilanchone, V., Beliakova-Bethell, N., Randall, A., DaSilva, R., Nagashima, K., McPherson, A., Baldi, P. and **Sandmeyer, S.B.** (2011) Ty3 spacer controls intracellular condensation and uncoating. J. Virol. 85, 3055-3066.
4. Irwin, B., Aye, M., Baldi, P., Beliakova-Bethell, N., Cheng, H., Dou, Y., Liou, W. and Sandmeyer, S.B. 2005. Retroviruses and yeast retrotransposons use overlapping sets of host genes. Gen. Res. **15**:641-654.
5. Beliakova-Bethell, N., Beckham, C., Giddings, T.H. Jr., Winey, M., Parker, R., and Sandmeyer, S. (2006) Virus-like particles of the Ty3 retrotransposon assemble in association with P-body components. RNA **12**:94-101

Five other significant products (yeast molecular biology and biochemistry)

6. Clemens*, K., Beliakova-Bethell*, N., Bilanchone, V., Larsen, L. Z., Nguyen, K., and **Sandmeyer, S. B.** Different sequence requirements for Ty3 RNA association with P-body proteins and packaging. (2013) Virus Research 171, 319-331.
7. Beliakova-Bethell, N., Terry, L.J., Bilanchone, V.W., DaSilva, R., Nagashima, K., Wente, S., and **Sandmeyer, S.B.** (2009). Ty3 nuclear entry is initiated by viruslike particle docking on nucleoporins. J. Virol. J. Virol. 83: 11914-11925. *Chosen for Nov Issue Spotlights.*

8. Larsen, L.S.Z., Beliakova-Bethell, N., Bilanchone,V., Zhang, M., Lamsa, A., DaSilva, R., Hatfield, G.W., Nagashima, K., and **Sandmeyer, S.B.** (2008). Ty3 nucleocapsid controls localization of particle assembly. J. Virol. 82:2501-2514
9. Kuznetsov, Y.G., Zhang, M., Menees, T. McPherson, A**,** and **Sandmeyer, S.** (2005) Atomic force microscopy investigation of the structure of Ty3 retrotransposon particles. J. Virol. **79**: 8032-8045.
10. Aye, M. Irwin, B., Archibald, H., and **Sandmeyer, S.B.** (2004) Host factors that affect Ty3 retrotransposition in *Saccharomyces cerevisiae*. Genetics 168, 1159-1176.

(d) Synergistic Activities

Lecturer in Explore Science on Saturdays Program [NSF FOCUS-Faculty Outreach Collaborations Uniting Scientists, Students and Schools (https://eee.uci.edu/11w/05450/eSOS2011/)] weekend lectures to students attending community colleges serving populations under-represented in the sciences.
Organization of undergraduate laboratory to investigate molecular biology of *Yarrowia*, an oleaginous yeast
Associate Director of Institute for Genomics and Bioinformatics (recipient of NLM training grant for bioinformatics and campus intercisciplinary research unit for bioinformatics)
Director of the UCI Genomics High-Throughput Facility (1999-present).
Chair, Department of Biological Chemistry (1997-2005).
Co-Chair of Cold Spring Harbor Retroviruses Meeting (2003).
Member, National Cancer Institute, Division of Basic Sciences, Board of Scientific Counselors (1998-2003)
Chair, Senior Editors, *Genetics* (2006-2007).
Organizer, UCI Mini-Symposia: Mobile DNA 02/19/10; 02/2011 HTS to P4 Medicine

(e) Collaborators & Other Affiliations

i. Collaborators (past 48 months)

P. Baldi (U.C. Irvine); J. Briggs, EMBL, Heidelberg, Germany; N. DaSilva (UCI); G.W. Hatfield (UCI); L. Huang (UCI); M. Johnston (U. CO, Denver); R. Lathrop (U.C. Irvine); A. McPherson (U.C. Irvine); R. Mitra (Washington U., St. Louis, MO); K. Nagashima (SAIC, NCI Frederick); B. Shanks (ISU).

ii. Graduate Advisors and Postdoctoral Sponsors

Postdoctoral Advisor: Maynard Olson, (U WA, Seattle, WA)
Ph.D. Thesis Advisor: Paul Bornstein, (U WA, Seattle, WA)

ii. Thesis Advisor and Postgraduate-Scholar Sponsor

Ph.D. --Lori Hansen, Director, Program and Alliance Management at Seattle Genetics; Douglas Chalker, Associate Professor, Dept Biology, Washington University; Philip Kinsey, DNA Technical Leader, State of MT; Jacqueline Kirchner, Project Manager, Amgen, Seattle, WA; Charles Connolly, Research Staff, University of Washington; Kathryn Orlinsky, homemaker; Jonathan Claypool, Patent attorney, Claypool Intellectual Property, Seattle, WA ; Jirong Gu, Product Manager for BioSolutions, Varian, Inc. Irvine, CA; Sophia Lin, MD/PhD, Pediatric Physician, Children's Hospital Orange County; Lynn Yieh, Bioinformatician, Johnson and Johnson, San Diego, CA; Henrietta Nymark, Invitrogen Inc., San Diego, CA, now homemaker; Liza Zicker-Larsen (2002-2006 Staff Scientist, CODA Genomics Inc., Laguna Hills, CA); Min Zhang (2002-2007) postdoctoral fellow in virology, UCLA; Nadia Beliokova-Bethell, Postdoctoral fellow, Professor Christopher Woelk, UCSD, San Diego, CA; Michael Aye, Ph.D. Postdoctoral Fellow, David Allis, U. VA; Liza Zicker-Larsen (Postdoc, 2007 Staff Scientist Verdezyne, Inc., Carlsbad, CA); Kim Nguyen (2004-2010) Postdoctoral Fellow; Daniel Voytas, U. MN
Past postdoctoral fellows--Virginia Bilanchone, Project scientist, UCI, Biological Chemistry*;* Douglas Forrest, Staff Scientist, US FDA*;* Thomas Menees, Professor, University of MO, Kansas City, MO*;* Joerg Hoffman, Senior Scientist, Division of Biochemistry, Friedrich-Alexander University Erlangen-Nurnberg Sandra Dildine, unknown; Victoria Perreau, Senior Research Fellow, Centre for Neuroscience, U of Melbourne, Australia; Michael Aye, Project Manager, Focus Diagnostics, Irvine, CA; Fang Fang, Staff Scientist, U. WA, Seattle, WA; Tarek Najdi, Bioinformatician, British Petroleum, San Diego, CA;Xiaojie Qi, Senior Scientist, Fred Hutchinson Cancer Center, Seattle, WA.
Current Students--Kristina Christiansen-Clemens*;* James Yu*;* Kurt Patterson
Current Postdoctoral Associates: Ivan Chang ; total advisees, 33.

KLAUS SCHMIDT-ROHR
Professor, Dept. of Chemistry, Iowa State University, Ames, IA 50011
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(a) Professional Preparation

Heidelberg & Mainz Universities, Germany	Physics	Diploma, 1989
Mainz University/Max-Planck Institute for Polymer Research, Germany	Physics	Ph.D., 1991
Multidimensional NMR Methods for the Investigation of Dynamics, Structure, & Order in Solid Polymers.		
University of California at Berkeley	Physical Chemistry	1993 – 1994

(b) Appointments

2005-present	Full Professor, Dept. of Chemistry, Iowa State University, Ames, IA
2000-2004	Associate Professor, Dept. of Chemistry, Iowa State University, Ames, IA
1997–1999	Associate Professor with tenure, Dept. of Polymer Science and Engineering, University of Massachusetts at Amherst
1995–1997	Assistant Professor, Dept. of Polymer Science and Engineering, UMass Amherst
1993–1994	Postdoctoral Research Fellow, Dept. of Chemistry, UC Berkeley, with A. Pines
1992	Project Manager, Max-Planck Institute (MPI) for Polymer Research
1991 – 1992	Staff Scientist, MPI for Polymer Research, laboratory of H. W. Spiess

(c) Products

i. Five products most closely related to the proposed project

1. H. N. Pham, A. E. Anderson, R. L. Johnson, K. Schmidt-Rohr, A. K. Datye, "Improved Hydrothermal Stability of Mesoporous Oxides for Aqueous Phase Reactions" *Angew. Chemie Int. Ed.* **51**, 13163-13167 (2012).
2. J-D. Mao, R. L. Johnson, J. Lehmann, D. C. Olk, E. G. Neves, M. L. Thompson, K. Schmidt-Rohr "Abundant and Stable Char in Soil: Implications for Soil Fertility and Carbon Sequestration", *Environ. Sci. Technol.* **46**, 9571-6 (2012).
3. C. E. Brewer, Y-Y. Hu, K. Schmidt-Rohr, S. D. Joseph, T. E. Loynachan, R. C. Brown, "Characteristics of the extent of pyrolysis for corn stover fast pyrolysis biochars", *J. Environm. Quality* **41**, 1115-1122 (2011).
4. C. E. Brewer, K. Schmidt-Rohr, J. A. Satrio, R. C. Brown, "Characterization of Biochar from Fast Pyrolysis and Gasification Systems" *Environ. Progr. Sust. Energy* **28**, 386-396 (2009).
5. J-D. Mao, K. Schmidt-Rohr, "Accurate Quantification of Aromaticity and Nonprotonated Aromatic Carbon Fraction in Natural Organic Matter by ^{13}C Solid State Nuclear Magnetic Resonance", *Environ. Sci. Technol*. **38**, 2680-2684 (2004).

ii. Five other significant products

1. X-W. Fang, K. Schmidt-Rohr, "Alkyl and Other Major Structures Formed in Model Maillard Reactions Studied by Solid-State NMR"**,** *J. Agri. Food Chem.* **59**, 481-490 (2011).
2. Y.-Y. Hu, A. Rawal, K. Schmidt-Rohr, "Strongly bound citrate stabilizes the apatite nanocrystals in bone", *Proc. Natl. Acad. Sci. USA* **107**, 22425-22429 (2010).

3. J-D. Mao, X-W. Fang, Y. Lan, A. Schimmelmann, M. Mastalerz, L. Xu, K. Schmidt-Rohr, "Chemical and nanometer structure of kerogen and its change during thermal maturation investigated by advanced solid-state ^{13}C NMR spectroscopy", *Geochim. Cosmochim. Acta* **74**, 2110-2127 (2010).
4. X-W. Fang , T. Chua, K. Schmidt-Rohr, M. L. Thompson**,** "Quantitative ^{13}C NMR of Whole and Fractionated Iowa Mollisols for Assessment of Organic Matter Composition", *Geochim. Cosmochim. Acta* **74**, 584-598 (2010).
5. K. Schmidt-Rohr, J-D Mao, D.C. Olk, "Nitrogen-Bonded Aromatics in Soil Organic Matter and Their Implications for a Yield Decline in Intensive Rice Cropping", *Proc. Nat. Acad. Sci.* **101**, 6351-6354 (2004).

(d) Synergistic Activities

- Associate Editor of *Organic Geochemistry*, 2002-2005
- Member of the Editorial Advisory Boards of *Chemistry of Materials* and *Journal of Magnetic Resonance*

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

M. Akinc (Iowa State), R. C. Brown (Iowa State), M. A. Chappell (USACE), B. C. Cook (Ames Lab), A. K. Datye (New Mexico), J. P. Heremans (Ohio State), M. Hong (ISU), M. G. Kanatzidis (Northwestern), D. A. Laird (ISU), J. Lehmann (Cornell), S. Mallapragada (Iowa State), J-D. Mao (Old Dominion), D. M. McKnight (CO-Boulder), R. B. Moore (Virgina Tech), K. Müllen (MPI Mainz), D. C. Olk (Natl. Soil Tilth Lab), J. Otaigbe (USMiss), E. M. Perdue (Ball State), J. J. Pignatello (Yale), A. Schimmelmann (Indiana), R. Venkatasubramanian (RTI), B. H. Shanks (ISU), I. R. da Silva (Viçosa, Brazil), H. W. Spiess (MPI Mainz), M. L. Thompson (Iowa State).

ii. Graduate Advisor and Postdoctoral Sponsors

Professor Hans W. Spiess, Max-Planck Institute for Polymer Research, Mainz, Germany
Postgraduate advisor: Professor Alexander Pines, Dept. of Chemistry, University of California, Berkeley CA

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

Graduate Students:
Current (3): Mr. Robert L. Johnson; Ms. Jinfang Cui; Mr. Keith Fritzsching
Past (12): Mr. Wei-Guo Hu (1999), Mr. Ioannis S. Polios (1997), Mr. Matthew G. Dunbar (1999), Mr. Douglas J. Harris (1999), Mr. Mikhail Y. Gelfer (1999), Mr. Daniel Mowery (2002, Florida State), Ms. Gabriele Menges (2003), Mr. Qiang Chen (2005; UNC), Mr. Aditya Rawal (2007; Ames Lab), Mrs. Xiaowen Fang (2008; UW Madison), Mr. Xueqian Kong (2010; UC Berkeley), Ms. Yanyan Hu (2011; Stony Brook)
Past Visiting (3): Mr. Kay Saalwächter, Mr. Eduardo R. deAzevedo, Mr. Fábio Becker-Guédes
Postdoctoral Associates /Visiting Scientists (10):
Dick Sandström (Stockholm), Kristin K. Kumashiro (Hawaii), Hironori Kaji (Kyoto, Japan), Tito J. Bonagamba (Sao Carlos, Brazil), Jingdong Mao (Rocky Mountain College), Detlef Reichert (Halle, Germany), Shengshu Hou (Nat'l Cheng Kung Univ., Taiwan), Evgenii Levin (Ames Lab), Mrs. Bosiljka Njegic, Mr. Aditya Rawal (New South Wales).

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(a) Education

Iowa State University	Chemical Engineering	B.S., 1983
California Institute of Technology	Chemical Engineering	M.S., 1985
California Institute of Technology	Chemical Engineering	Ph.D., 1988

(b) Appointments

2010 – present	Steffenson Professor, Chemical & Biological Engineering, Iowa State University
2008 – present	Director, NSF Engineering Research Center for Biorenewable Chemicals, Iowa State University
2007 – 2010	Professor, Chemical & Biological Engineering, Iowa State University
1999 – 2007	Associate Professor, Chemical & Biological Engineering, Iowa State Univ.
1997 – 1999	Research Department Manager, Shell Chemical Company, Houston, TX
1988 - 1997	Research Engineer, Shell Chemical Company, Houston, TX

(c) Products

i. Five Products Most Closely Related to the Proposed Project

1. Wang, T., Combs, E., Pagan-Torres, Y.J., Dumesic, J.A., and Shanks, B.H., "Water-compatible Lewis acid-catalyzed conversion of carbohydrates to 5-hydroxymethylfurfural in a biphasic solvent system," *Top. Catal.*, **55**, 657-662 (2012).
2. Chia, M., Schwartz, T.J., Shanks, B.H., and Dumesic, J.A., "Triacetic Acid Lactone as a Biorenewable Platform Chemical," *Green Chem.,* **14**, 1850-1853 (2012).
3. Pagan-Torres, Y.J., Wang, T., Gallo, J.M.R., Shanks, B.H., and Dumesic, J.A., "Production of 5-Hydroxymethylfurfural from Glucose Using a Combination of Lewis and Brønsted Acid Catalysts in Water in a Biphasic Reactor with an Alkylphenol Solvent," *ACS Catal.*, **2,** 930-934 (2012).
4. Snell, R.W., Combs, E. and Shanks, B.H., "Aldol Condensations using Bio-oil Model Compounds: The Role of Acid-Base Bi-functionality," *Catal. Today,* **53**, 1248-1253 (2010).
5. Tang, Y., Miao, S., Shanks, B.H., and Zheng, X., "Bifunctional Mesoporous Organic–Inorganic Hybrid Silica for Combined One-step Hydrogenation Esterification," *Appl. Catal A: Gen.,* **375**, 310-317 (2010).

ii. Five Other Significant Products

6. Deutsch, K.L., Lahr, D.G., and Shanks, B.H., "Probing the ruthenium-catalyzed higher polyol hydrogenolysis reaction through the use of stereoisomers," *Green Chem.*, **14**, 1635-1642 (2012).
7. Deutsch, K.L. and Shanks, B.H., "Active Species of Copper Chromite Catalyst in C-O Hydrogenolysis of 5-Methylfurfuryl Alcohol," *J. Catal,.* **285**, 235-241 (2012).
8. Miao, S. and Shanks, B.H., "On the Mechanism of Acetic Acid Esterification over Sulfonic Acid Functionalized Mesoporous Silica," *J. Catal.*, **279**, 136-143 (2011).
9. Cinlar, B. and Shanks, B.H., "Characterization of the Acidic Sites in Organic Acid Functionalized Mesoporous Silica in the Aqueous Phase," *Appl. Catal. A: Gen.,* **396**, 76-84 (2011).

10. Shanks, B.H., "Conversion of Biorenewable Feedstocks: New Challenges in Heterogeneous Catalysis," *Ind. Eng. Chem. Res.,* **49**, 10212-10217 (2010).

(d) Synergistic Activities

Lecturer, Energy and Materials from the Sun Summer School, Rolduc Abbey, Netherlands, June 2011
Advisory Board, Wi(PR)$_2$EM, University of Puerto Rico – Mayaguez, 2009-present
Lecturer, International Workshop on Biorenewables held in Seeon, Germany, August 2010.
Organizing Committee, NSF Workshop on Breaking the Chemical & Engineering Barriers to Lignocellulosic Biofuels, June 25-26, 2007.
Co-taught, Workshops on Biodiesel Technology, >600 national and international students and professionals, 2003-09.

(e) Collaborators & Other Affiliations

Collaborators (last 48 months)

James Dumesic, Chemical and Biological Engineering, University of Wisconsin
Matt Neurock, Robert Davis, Chemical Engineering, University of Virginia
Abhaya Datye, Chemical Engineering, University of New Mexico
Bert Chandler, Chemistry, Trinity University
Linda Broadbelt, Chemical Engineering, Northwestern University
Robert Brown, Ted Heindel, Mechanical Engineering, ISU
George Kraus, Klaus Schmidt-Rohr, Keith Woo, Chemistry, ISU
Basil Nikolau, Biochemistry, ISU
Ka-Yiu San, Ramon Gonzalez, Chemical Engineering, Rice University
Nancy Da Silva, Chemical Engineering, U. California, Irvine
Tony Dean, Chemical Engineering, Colorado School of Mines
Yuriy Roman, Massachusetts Institute of Technology

Graduate Advisor

James E. Bailey (deceased)

Thesis Advisor and Postgraduate-Scholar Sponsor (last 5 years)

Past Students: Karl Albrecht (Ph.D. – 2008, Pacific Northwest National Laboratory), Sarah Hruby (Ph.D. – 2009, Dow Company), Sikander Hakim (Ph.D. – 2009, University of Wisconsin), Zheng Li (Ph.D. – 2009, University of Michigan), Nattaporn Lohitharn (Postdoc – 2009, Logos Technologies); Shaoju Miao (Postdoc – 2010, Shell); Basak Cinlar (Ph.D. – 2010, DSM); Dursan Ozcan (M.S. – 2010, University of Edinburgh); Pushkaraj Patwardhan (Ph.D. – 2010, BASF); Pedro Ortiz-Toral (Ph.D. – 2011, Gas Technology Institute); Keenan Deutsch (Ph.D. – 2012, BASF); Ryan Snell (Ph.D. – 2012, ChevronPhillips)
Total # of graduate students/postdocs advised: 23/7.
Current Students: Jason Anderson, Yongsuck Choi, Michael Nolan, Michael Nolte, Tianfu Wang, Jing Zhang, Umayangani Wanninayake, Hugh Warren

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(a) Professional Preparation

Iowa State University	Chemical Engineering	B.S., 1983
California Institute of Technology	Chemical Engineering	Ph.D., 1989

(b) Appointments

2010-present	Thrust 2 Leader, NSF Center for Biorenewable Chemicals (CBiRC), ISU
2009-present	Manley R. Hoppe Professor, Department of Chemical and Biological Engineering, Iowa State University
2008–2010	Thrust 2 Co-Leader, NSF Center for Biorenewable Chemicals (CBiRC), ISU Professor, Department of Chemical and Biological Engineering, Iowa State University
1999 – present	Adjunct Professor, Department of Bioengineering, Rice University
1999 – 2009	Professor, Chemical Engineering, Iowa State University
1999	Professor, Bioengineering and Chemical Engineering, Rice University
1997 – 1999	Associate Professor, Bioengineering, Rice University
1993 – 1999	Associate Professor, Chemical Engineering, Rice University
1988 – 1993	Assistant Professor, Chemical Engineering, Rice University

(c) Products (Selected from > 80 peer-reviewed publications)

i. Five products most closely related to the proposed project

1. Yoon, J.M., Zhao, L. and J.V. Shanks, "Metabolic Engineering with Plants for a Sustainable Biobased Economy**,** *Annual Review of Chemical and Biomolecular Engineering*, Volume 4, In Press, 2013.
2. Ranganathan, S., Tee, T.W., Chowdhury, A., Zomorrodi, A.R., Yoon, J. M., Fu, Y. Shanks, J.V. and. Maranas, C.D.2012. "An Integrated Computational and Experimental Study for Overproducing Fatty Acids in *Escherichia coli*," *Met. Engr.* **14**, 687-704 DOI: 10.1016/j.ymben.2012.08.008.
3. Choudhary, M.K., Yoon, J.M., Gonzalez, R. and J.V. Shanks. 2011. Metabolic Fluxes of *Escherichia coli* in Anaerobic Fermentation of Glucose Using 2-dimensional Nuclear Magnetic Resonance (NMR) Spectroscopy. *Biotechnology and Bioprocess Engineering* **16,** 419-437.
4. Peebles, C.A.M., Sander, G. W., Hughes, E. H., Peacock, R., J.V. Shanks, and K.-Y. San.2011. The Expression of 1-deoxy-D-xylulose Synthase and Geraniol 10-hydroxylase or Anthranilate Synthase Increases Terpenoid Indole Alkaloid Accumulation in *Catharanthus roseus* Hairy Roots. *Metabolic Engineering* **13**, 234-240. http://dx.doi.org/10.1016/j.ymben.2010.11.005
5. Murarka, A., Clomburg, J. M., Moran, S., Shanks, J. V. and R. Gonzalez. 2010. Metabolic Analysis of Wild-Type *Escherichia coli* and a Pyruvate Dehydrogenase Complex (PDHC)-deficient derivative reveals the Role of PDHC in the Fermentative Metabolism of Glucose. *J. Biological Chemistry*,**285**, 31548-31558. doi:10.1074/jbc.M110.121095

ii. Five other significant products

1. Iyer, V.V., Sriram, G., Fulton, D. B., Zhou, R., Westgate, M.E., and Shanks, J.V. 2008. Metabolic Flux Maps Comparing the Effect of Temperature on Protein and Oil Biosynthesis in Developing Soybean Cotyledons. *Plant Cell and Environment*, 31 (4), 506–517. http://onlinelibrary.wiley.com/doi/10.1111/j.1365-3040.2008.01781.x/pdf

2. Sriram, G., D. B. Fulton, and Shanks, J. V. 2007. Flux Quantification in Central Carbon Metabolism of *Catharanthus roseus* Hairy Roots by ^{13}C Labeling and Comprehensive Bondomer Balancing. *Phytochemistry,* **68**, 2243-2257. *http://dx.doi.org/10.1016/j.phytochem.2007.04.009*
3. Sriram, G., Iyer, V. V., Fulton, D. B., and Shanks, J. V. 2007. Identification of Hexose Hydrolysis Products in Metabolic Flux Analytes: A Case Study of Levulinic acid in Plant Protein Hydrolysate. *Metabolic Engineering,* **9**, 442-451.
4. Sriram, G., Fulton, D. B., Iyer V., Peterson, J. M., Zhou, R., Westgate, M. E., Spalding, M. H. and Shanks, J. V. 2004. Quantification of Compartmented Metabolic Fluxes in Developing Soybean (*Glycine max*) Embryos by Employing Biosynthetically Directed Fractional ^{13}C Labeling, 2-D [^{13}C,^{1}H] NMR and Comprehensive Isotopomer Balancing. *Plant Physiol.* **136**: 3043-3057. www.plantphysiol.org/cgi/doi/10.1104/pp.104.050625
5. Sriram, G.and Shanks, J. V. 2004. Improvements in Metabolic Flux Analysis using Carbon Bond Labeling Experiments: Bondomer Balancing and Boolean Function Mapping. *Metabol. Eng,* **6**, 116-132. doi:10.1016/j.ymben.2004.02.003

(d) Synergistic Activities

Thrust 2 Leader, NSF Center for Biorenewable Chemicals (CBiRC), Iowa State University
DOE, The Office of Biological and Environmental Research (BER) Advisory Committee (BERAC) 2011- present
AIChE Food, Pharmaceutical and Bioengineering Division, Area 15c Plenary Award 2010
Editorial Board: *Biotechnology Progress*, *Current Opinion in Biotechnology , Metabolic Engineering*
Mentoring of over 60 undergraduate and high school researchers in engineering – of which over 65% are women and underrepresented minorities, (1988-present)
Mentoring of 2 female graduate students in Mech. Engr; of 3 CBE assistant professors (2 female, 1 URM)

(e) Collaborators & Other Affiliations (outside ISU)

Collaborators (past 48 months)

Nancy DaSilva, Chemical Engineering, University of California Irvine; John Everard, Dupont, Delaware Sue Gibson, Plant Biology, University of Minnesota; Ramon Gonzalez, Chemical and Biomolecular Engineering, Rice University; Harin Kanani, Pioneer Hybrid International; Costas Maranas, Penn State University; Govind S. Nadathur, Marine Sciences, University of Puerto Rico, Mayaguez; Ka-Yiu San, Bioengineering, Rice University; Suzanne Sandmeyer, Biological Chemistry, University of California Irvine; Gordon V. Wolfe, Biological Sciences, California State University, Chico ; Larry Wackett, Biochemistry, University of Minnesota

Graduate Advisor

James E. Bailey (deceased)

Thesis Advisor and Postgraduate-Scholar Sponsor (past 60 months)

Past Students (last 5 years and current position):
Madhuresh Choudhury (M.S. 2008, Indo-Gulf Fertilizer); Vidya Iyer (Ph.D. 2006, Bristol Myers Squibb) Guy Sander (Ph.D. 2009, Asst. Professor, Univ. of Minn. Duluth); Yanfen Fu (M.S. 2011, pre-doctoral student, Univ. of Washington)

Current Students:
Le Zhao, Mark Brown, Ting Wei Tee, Quyen Truong, Erin Boggess, BCB (co-advised by J.Dickerson)

Current Research Scientist: Jong Moon Yoon

Total number of graduate students advised and postdocs sponsored >30.

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(a) Professional Preparation

Nankai University	Biochemistry	B.S. & 2002
University of Illinois, Urbana-Champaign	Chemical and Biomolecular Engineering	Ph.D. & 2009
University of Illinois, Urbana-Champaign	Synthetic Biology	Apr. 2009–Mar. 2011

(b) Appointments

Jan. 2013 – Present	Assistant Professor, Department of Chemical and Biological Engineering, Iowa State University
Apr. 2011–Dec. 2012	Research Assistant Professor, Department of Chemical and Biomolecular Engineering, University of Illinois, Urbana-Champaign

(c) Products

i. Five products most closely related to the proposed project

1. Z. Shao and H. Zhao. "DNA Assembler: a Synthetic Biology Tool for Characterizing and Engineering Natural Product Gene Clusters." Methods in Enzymology 517, 203-24 (2012).

2. J. Du, Z. Shao, and H. Zhao, "Engineering Microbial Factories for Synthesis of Value-added Products." Journal of Industrial Microbiology and Biotechnology 38, 873-90 (2011).

3. Z. Shao, Y. Luo, and H. Zhao, "Rapid Characterization and Engineering of Natural Product Biosynthetic Pathways via DNA Assembler." Molecular Biosystems 7, 1056-9 (2011).

4. Z. Shao, H. Zhao, and H. Zhao, "DNA Assembler, an in vivo Genetic Method for Rapid Construction of Large Recombinant DNA." Nucleic Acids Research 37, e16 (2009).

5. Z. Shao, J. Blodgett, B. Circello, A. Eliot, R. Woodyer, G. Li, A. van der Donk, W. M. Metcalf, and H. Zhao, "Biosynthesis of 2-Hydroxyethylphosphonate, an Unexpected Intermediate Common to Multiple Phosphonate Biosynthetic Pathways." Journal of Biological Chemistry 283, 23161-8 (2008).

ii. Five other significant products

6. Z. Shao, Y. Luo, and H. Zhao. "DNA Assembler Method for Construction of Zeaxanthin Producing Strains of Saccharomyces cerevisiae." Methods in Molecular Biology 898, 251-62 (2012).

7. J. Sun, Z. Shao, H. Zhao, N. Nair, F. Wen, J. Xu, and H. Zhao, "Systematic Characterization of a Panel of Constitutive Promoters for Applications in Pathway Engineering in Saccharomyces cerevisiae." Biotechnology and Bioengineering doi: 10.1002/bit.24481 (2012).

8. R. Woodyer, Z. Shao, W. M. Metcalf, W. A. van der Donk, and H. Zhao, "Heterologous Production of Fosfomycin and Identification of the Minimal Fosfomycin Biosynthetic Cluster." Chemical & Biology 13, 1171-82 (2006).

9. D. Xie, Z. Shao, J. Achkar, W. Zha, J. W. Frost, and H. Zhao, "Microbial Synthesis of Triacetic Acid Lactone." Biotechnology and Bioengineering 93, 727-36 (2006).

10. W. Zha, Z. Shao, J. W. Frost, and H. Zhao, "Rational Pathway Engineering of Type I Fatty Acid Synthase Allows Biosynthesis of Triacetic Acid Lactone from D-Glucose in vivo." Journal of the American Chemical Society 126, 4534-5 (2004).

(d) Synergistic Activities

Dr. Shao is a new faculty who joined ISU on January 2013. In the course she taught, "Biochemical Engineering", she incorporated the most advanced development in protein, pathway and genome engineering into the subjects, and attempted to bring students to the cutting-edge technology frontier.

(e) Collaborators & Other Affiliations *(please list alphabetically in each section)*

i. Collaborators and Co-Editors (past 48 months)

Name	Organizational Affiliation
Eric W Cochran	Chemical and Biological Engineering, Iowa State University

ii. Graduate Advisors and Postdoctoral Sponsors

Name	Organizational Affiliation
Huimin Zhao	Chemical and Biomolecular Engineering, University of Illinois, Urbana-Champaign

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

Name	Organizational Affiliation
Jose M Suastegui-Pastrana	Chemical and Biological Engineering, Iowa State University
Mingfeng Cao	Chemical and Biological Engineering, Iowa State University (postdoc)

1/1 = Total number of graduate students advised and postdocs sponsored

JEAN-PHILIPPE TESSONNIER
Assistant Professor, Chemical & Biological Engineering Department
Iowa State University
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(a) Professional Preparation

University of Strasbourg	Chemistry	B.S., 1999
University of Strasbourg	Materials Chemistry	M.S., 2001
University of Strasbourg	Chemistry - Catalysis	Ph.D., 2005
Fritz Haber Institute	Catalysis	Postdoc, 2005-2007

(b) Appointments

2012 – present	Assistant Professor and Carol & Jack Johnson Faculty Fellow, Chemical & Biological Engineering, Iowa State University
2012 – present	Associate Scientist, U.S. Department of Energy, Ames Laboratory
2012 – present	Co-Investigator, NSF Engineering Research Center for Biorenewable Chemicals
2011 – 2012	Visiting Researcher, Chemical & Biomolecular Engineering, University of Delaware
2008 – 2010	Project Leader, Fritz Haber Institute of the Max Planck Society, Berlin, Germany

(c) Products

(i) Five closely related products

1. Mette, K., Bergmann, A., Tessonnier, J.-P., Hävecker, M., Yao, L., Ressler, T., Schlögl, R., Strasser, P., and Behrens, M., Nanostructured Manganese Oxide Supported on Carbon Nanotubes for Electrocatalytic Water Splitting, *ChemCatChem* **2012**, 4, 851-862.
2. Arrigo, R., Schuster, M. E., Wrabetz, S., Girgsdies, F., Tessonnier, J.-P., Centi, G., Perathoner, S., Su, D. S., and Schlögl, R., New Insights from Microcalorimetry on the FeO_x/CNT-Based Electrocatalysts Active in the Conversion of CO_2 to Fuels, *ChemSusChem* **2012**, 5, 577-586.
3. Villa, A., Tessonnier, J.-P., Majoulet, O., Su, D. S., and Schlögl, R., Transesterification of Triglycerides Using Nitrogen-Functionalized Carbon Nanotubes, *ChemSusChem* **2010**, 3, 241-245.
4. Villa, A., Tessonnier, J.-P., Majoulet, O., Su, D. S., and Schlögl, R., Amino-Functionalized Carbon Nanotubes as Solid Basic Catalysts for the Transesterification of Triglycerides, *Chem. Commun.* **2009**, 4405-4407.
5. Tessonnier, J.-P., Villa, A., Majoulet, O., Su, D. S., and Schlögl, R., Defect-Mediated Functionalization of Carbon Nanotubes as a Route to Design Single-Site Basic Heterogeneous Catalysts for Biomass Conversion, *Angew. Chem. Int. Ed.* **2009**, 48, 6543-6546.

(ii) Five other significant products

1. Tessonnier, J.-P., and Barteau, M. A., Dispersion of Alkyl-Chain-Functionalized Reduced Graphene Oxide Sheets in Nonpolar Solvents, *Langmuir* **2012**, 28, 6691-6697.
2. Marichy, C., Tessonnier, J.-P., Ferro, M. C., Lee, K.-H., Schlögl, R., Pinna, N., and Willinger, M.-G., Labeling and Monitoring the Distribution of Anchoring Sites on Functionalized CNTs by Atomic Layer Deposition, *J. Mater. Chem.* **2012**, 22, 7323-7330.
3. Tessonnier, J.-P., and Su, D. S., Recent Progress on the Growth Mechanism of Carbon Nanotubes: A Review, *ChemSusChem* **2011**, 4, 824-847.

4. Rinaldi, A., Tessonnier, J.-P., Schuster, M. E., Blume, R., Girgsdies, F., Zhang, Q., Jacob, T., Abd Hamid, S. B., Su, D. S., and Schlögl, R., Dissolved Carbon Controls the Initial Stages of Nanocarbon Growth, *Angew. Chem. Int. Ed.* **2011**, 50, 3313-3317.
5. Tessonnier, J.-P., Rosenthal, D., Girgsdies, F., Amadou, J., Begin, D., Pham-Huu, C., Su, D. S., and Schlögl, R., Influence of the Graphitisation of Hollow Carbon Nanofibers on Their Functionalisation and Subsequent Filling with Metal Nanoparticles, *Chem. Commun.* **2009**, 7158-7160.

(d) Synergistic Activities

Member of the scientific committee, CarboCat V, Bressanone-Brixen, Italy, June 28-30, 2012.
Associate Editor, Journal of Nanoparticle Research, 2012 – present.
Editorial board peer-review member, ISRN Nanotechnology, 2011 – present.
Guest Lecturer, "Modern Methods in Heterogeneous Catalysis Research" lecture series, held at the Fritz Haber Institute of the Max Planck Society, Berlin, Germany. Multiple lectures on Biomass Conversion (2010), Micro- and Mesoporous Materials (2010), Carbon Materials in Heterogeneous Catalysis (2009).

(e) Collaborators & Other Affiliations

Collaborators (last 48 months)

Mark A. Barteau, Chemical Engineering, University of Michigan
Gabriele Centi, Industrial Chemistry & Materials Engineering, University of Messina, Italy
Ovidiu Ersen, IPCMS, University of Strasbourg, France
Timo Jacob, Electrochemistry, University of Ulm, Germany
Benoit Louis, Jean Sommer, Chemistry, University of Strasbourg, France
Martin Muhler, Industrial Chemistry, Ruhr-Universität Bochum, Germany
Dmitry Murzin, Åbo Akademi University, Finland
Marcelo Maciel Pereira, Chemistry, Universidade Federal do Rio de Janeiro, Brazil
Cuong Pham-Huu, LMSPC, University of Strasbourg, France
Nicola Pinna, Chemistry, Humboldt-Universität zu Berlin, Germany
Thorsten Ressler, Chemistry, TU Berlin, Germany
Robert Schlögl, Fritz Haber Institute of the Max Planck Society, Germany
Peter Strasser, Chemistry, TU Berlin, Germany
Yushan Yan, Chemical & Biomolecular Engineering, University of Delaware

Advisor

Ph.D. advisor: Marc-Jacques Ledoux (University of Strasbourg), Postdoctoral advisor: Robert Schlögl (Fritz Haber Institute).

Thesis Advisor and Postgraduate-Scholar Sponsor (last 5 years)

Past Students: Alberto Villa (Postdoc - 2009, University of Milan), Li-De Yao (Postdoc - 2010, Aalto University), Ali Rinaldi (Ph.D. - 2010, co-advised with Robert Schlögl, Nanyang Technological University), Katharina Mette (M.S. - 2010, Fritz Haber Institute).
Total # of graduate students/postdocs advised: 3/2.

Current Students: Alex Liu, John Matthiesen

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(a) Professional Preparation

Harvey Mudd College	Chemistry	B.S., 1977
Stanford University	Chemistry	Ph.D., 1984
University of Wisconsin-Madison	Chemistry	1984-1986

(b) Appointments

2004-present	Associate Chair, Department of Chemistry
2003-present	Professor, Department of Chemistry, Iowa State University
1992-2003	Associate Professor, Department of Chemistry, Iowa State University
1986-1992	Assistant Professor, Department of Chemistry, Iowa State University

(c) Products

i. Five products most closely related to the proposed project

1. Roberts, G. M.; Pierce, P. J.; Woo, L. K. "Pd Complexes with *N*-heterocyclic Carbene Ligands as Catalysts for the Alkoxycarbonylation of Olefins." *Organometallics*, **2013**, Accepted. http://dx.doi.org/10.1021/om300959f
2. Anding, B. J.; Brgoch, J.; Miller, G. J.; Woo, L. K. "C–H Insertion Catalyzed by Tetratolylporphyrinato Methyliridium via a Metal-Carbene Intermediate" *Organometallics* **2012**, *31*, 5586–5590. http://dx.doi.org/10.1021/om3005433
3. Anding, B. J.; Ellern, A.; Woo, L. K. "Olefin Cyclopropanation Catalyzed by Iridium(III) Porphyrin Complexes." *Organometallics* **2012**, *31*, 3628-3635. http://dx.doi.org/10.1021/om300135f
4. Klobukowski, E. R.; Angelici, R. J.; Woo, L. K. "Bulk Gold-Catalyzed Reactions of Isocyanides, Amines, and Amine N-Oxides." *Organometallics* **2012**, *31*, *31*, 2785-2792. http://dx.doi.org/10.1021/om201068g
5. Klobukowski, E. R.; Angelici, R. J.; Woo, L. K. "Bulk Gold Catalyzed Oxidations of Amines and Benzyl Alcohol Using Amine N-Oxides as Oxidants." *Catal. Lett.* **2012**, *142*, 161-167. http://dx.doi.org/10.1007/s10562-011-0758-0

ii. Five other significant products

1. Bagherzadeh, M.; Zare, M.; Amani, V.; Ellern, A.; Woo, L. K. "Dioxo and oxo-peroxo molybdenum(VI) complexes bearing salicylidene 2-picoloyl hydrazone: structures and catalytic performances" *Polyhedron.* Accepted. http://dx.doi.org/10.1016/j.poly.2013.01.054
2. Amini, M.; Bagherzadeh, M.; Moradi-Shoeili, Z.; Boghaei, D. M.; Ellern, A.; Woo, L. K. "Selective oxidation of sulfides and olefins by a manganese(III) complex containing an N,O-type bidentate oxazine ligand" *J. Coor. Chem.* **2013**, *66*, 464-472. http://dx.doi.org/10.1080/00958972.2012.761339
3. Bagherzadeh, M.; Amini, M.; Ellern, A.; Woo, L. K. "Catalytic Efficiency of a Novel Complex of Oxoperoxo Molybdenum(VI): Synthesis, X-ray Structure and Alkane Oxidation." *Inorg. Chem. Commun*. **2012**, *15*, 52-55. http://dx.doi.org/10.1016/j.inoche.2011.09.037

4. Klobukowski, E. R.; Mueller, M.; Angelici, Woo, L. K. "Conversions of Cyclic Amines to Nylon Precursor Lactams Using Bulk Gold and Fumed Silica Catalysts." *ACS Catalysis*, **2011**, *1*, 703-708. http://dx.doi.org/10.1021/cs200120c
5. Zhou, Y.; Ryu, E.-H.; Zhao, Y.; Woo, L. K. "Solvent Responsive Metalloporphyrins: Binding and Catalysis." *Organometallics*, 2007, *26*, 358-364. http://dx.doi.org/10.1021/om060791z

(d) Synergistic Activities

1. Mentor to NSF REU undergraduate researchers
2. Senior Personnel in NSF ERC (2008-2013)
3. Mentor to NSF RET middle school teachers
4. Member of Faculty Advisory Board of ISU Bioeconomy Institute
5. HHMI participant for transforming undergraduate teaching laboratories

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Robert J. Angelici	Iowa State University
Andrew Hillier	Iowa State University
Yan Zhao	Iowa State University

ii. Graduate Advisors and Postdoctoral Sponsors

Charles P. Casey	University of Wisconsin-Madison
James P. Collman	Stanford University

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

BJ Anding	Iowa State University
Mojtaba Bagherzadeh	Visiting Professor, Sharif University of Technology, Iran
Taiwo Dairo	Iowa State University
Wenya Lu	Postdoc, Iowa State University
Erik Klobukowski	Ph.D., Iowa State University
Harun M. Mbuvi	Ph.D., Iowa State University
Gina Roberts	Iowa State University
Yibo Zhou	Postdoc, Iowa State University

35 = Total number of graduate students advised and postdocs sponsored

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(a) Professional Preparation

U.C. Santa Cruz	Biology	B.S., 1971
U.C. Los Angeles	Biology	Ph.D., 1980

(b) Appointments

1998-present	Professor, GDCB, Iowa State University
1995-1998	Associate Professor, Botany, Iowa State University
1990-1995	Assistant Professor, Botany, Iowa State University
1983-1988	Senior Research Scientist, Cell Biology Division, NPI, Inc.
1980-1983	Postdoctoral Fellow, Biochemistry, U.C. Davis

(c) Products (of 150 total)

(i) Five most closely related to proposal

Hur M, Cambell AA, Almeida-de-Macedo M, Li L, Ransom N, Jose A, Nikolau BJ, **Wurtele ES**. 2013. A global approach to analysis and interpretation of metabolic data for plant natural product discovery. Natural Product Reports. Feb, DOI:10.1039/C3NP20111B

Yeo YS, S. Nybo E, Chittiboyina AG, Weerasooriya AD, Wang YH, Góngora-Castillo E, Vaillancourt B, Buell CR, DellaPenna D, Celiz MD, Jones AD, Wurtele ES, Ransom N, Dudareva N, Shabaan KA, Tibrewal N, Chandra S, Smillie T, Khan IA, Coates RM, Watt DS, Chappell J. 2012. Functional identification of valerena-1,10-diene synthase, a terpene synthase catalyzing a unique chemical cascade in the biosynthesis of biologically active sesquiterpenes in *Valeriana officinalis*. J Biol Chem. *doi:10.1074*

Ngaki MN, Louie GV, RN, Manning G, Pojer F, Bowman ME, Li L, Elise Larsen E, **Wurtele ES**, Noel JP. 2012. Evolution of the chalcone isomerase fold from fatty acid-binding to stereospecific enzyme. Nature. 485:530-533. doi:10.1038/nature11009

Feng YP, Hurst J. Almeida-De-Macedo M. Chen X. Li L. Ransom N, **Wurtele ES**. 2012. A massive human co-expression-network and its medical applications. *Special Issue: Molecular Networks and Disease*. Systems Biology, Chemistry & Biodiversity. May;9(5):868-87. doi: 10.1002/cbdv.201100355

Wurtele ES, Chappell J, Jones AD, Celiz MD, Ransom N, Hur M, Rizshsky L, Dixon P Liu J, Widrlechner MP, Nikolau BJ. 2012. Medicinal Plants: A Public Resource for Metabolomics and Hypothesis Development. Metabolites. *2*(4), 1031-1059; doi:10.3390/metabo2041031 -

(ii) Five other significant products

Feng YP, Hurst J. Almeida-De-Macedo M. Chen X. Li L. Ransom N, **Wurtele ES**. 2012. A massive human co-expression-network and its medical applications. *Special Issue: Molecular Networks and Disease*. Systems Biology, Chemistry & Biodiversity. 9:868-87. doi: 10.1002/cbdv.201100355

Quanbeck S...(13 others).. **Wurtele ES**, Nikolau .BJ. 2012. Metabolomics as a hypothesis-generating functional genomics tool for the annotation of Arabidopsis thaliana genes of "unknown function" Frontiers of. Plant Science. doi: 10.3389/fpls.2012.00015

Li L, Foster C, Gan Q, Nettleton D, James MG, Myers AM, **Wurtele ES**.2009. Identification of the novel protein QQS as a component of the starch metabolic network in Arabidopsis leaves. Plant Journal. Online 2/09 http://www3.interscience.wiley.com/cgi-bin/fulltext/121641416/HTMLSTART

Mentzen W, **Wurtele ES**. 2008. Regulon Organization of Arabidopsis. BMC Plant Biology. 8:99 Sept 30 http://www.biomedcentral.com/1471-2229/8/99 highly accessed article

(d) Synergistic Activities

1. Bioinformatics software and databases
    - PMR database and analysis for combined metabolomics and transcriptomics of plants and microorganisms. http://metnetdb.org/PMR/
    - MedicinalPlantsMetabolomics Resource: http://metnetdb.org/mpmr_public/
    - MetNet Database and Software for Bioinfomatics: http://metnetdb.org/
    - Meta!Blast videogame for metabolic biology: http://metablast.org
2. Director *Meta!Blast: an interactive virtual metabolic cell videogame* ABC5 (Iowa) (5/07); Canada NOVA (8/07); emerging technologies segment CNN (12/08); Second Place, Chlorofilms 2010; Finalist, Learning Lab, (top 5% of >1000 submissions) MacArthur Foundation, 2010; SciVis International Visualization Challenge winner, *Science/NSF* 2012
3. META!BLAST EXHIBITS: *Museums of the National Council of Science Museums of India http://www.ncsm.org.in/*

   November 2009-present: MetaBlast posters for cell and metabolic biology. Birla Industrial & Technological Museum, Kolkata (Headquarters); Science City, Kolkata (1.5 million visitors/y) (permanent exhibit in Rotunda); Biotechnology Gallery, Visvesvaraya Industrial & Technological Museum, Bangalore; Nehru Science Centre, Mumbai; NCSM Local and Regional Science Centers: Kalimpong, Gangtok, Siliguri, Guwahati, Assam

   November-December, 2012-present. Meta!Blast computer game. Module: The Cell. Science City, Kolkata (1.5 million visitors/y) (permanent exhibit, established in NanoScience Lab as a team-based computer game for high school students, and in main Rotunda as a single-player interactive exhibit for the public); established as single-player interactive exhibit for the public at: NCSM Regional Science Museum, Callicut; Karala State Science and Technology Museum,Thiruvananthapuram, and NCSM District Science Museum, Tirunelveli
4. Organizer: Metabolic Networking in Plants, 1999; Third International Congress on Plant Metabolomics, 2004. Funded by NSF, USDA, and DOE
5. Invited workshops 2009-present: MetNet/PMR tools for computational biology (9); Meta!Blast (20)
6. Co-Editor, *Concepts in Plant Metabolomics* (2007). 21-chapters. Springer Press (ISBN-10: 1-4020-5607-9).
7. Editorial Board: BMC Plant Biology; Journal of Botany
8. Panel/Study Sections. National Science Foundation, Metabolic Biochemistry (six), Arabidopsis 2010 (one), Interagency Systems Biology (one), Systems Biology (one). USDA, Biochemistry (one). National Institutes of Health: Modeling and Analysis of Biological Systems (study section: seven panels), NIAID Science Education Award (one); NIH-SEPA (two).

(e) Collaborators & Other Affiliations

i. Collaborators (past 48 months)

Myers, Alan (ISU), Nielsen-Hamilton, M. (ISU), Ohlrogge, John (Michigan State Univ.), Oliver, D. J. (ISU), Reinot, Andres (ISU), Dickerson, Julie (ISU/NSF), Rhee, Sue (Stanford Univ.), Schnable, Patrick (ISU), Shanks, Jackie (ISU), Spalding, Martin (ISU), Sumner, L. W. (Nobel Foundation), Campbell, Alexis (ISU), Bassham, Diane (ISU)

ii. Graduate and Postdoctoral Advisors

Bernard Phinney (UCLA)deceased, Eric Conn (UC-Davis)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

Heather Babka, Suh-Yeon Choi, Matthew Hillwig, Jie Li, Li Ling, Lankun Wu, Micheline Ngaki, Yves Sucaet, Jon Hurst, Yaping Weng, Matt Crispin, Dallas Jones, Manhoi Hur, Mandela Magnidjem, Marcia Macedo.

Total graduate students/postdocs: 39

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